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**REPORT OF THE
NORTHERN REGIONAL RESEARCH CENTER**

June 1980

PROCUREMENT
CURRENT SERIAL RECORDS

JAN 28 '83

U.S. DEPT. OF AGRICULTURE
NORTH CENTRAL REGION
JAN 28 1983

North Central Region
Agricultural Research
Science and Education Administration
UNITED STATES DEPARTMENT OF AGRICULTURE

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PROGRESS REPORT
OF THE
NORTHERN REGIONAL RESEARCH CENTER
JUNE 1980

INTRODUCTION

The Northern Regional Research Center, located at Peoria, Illinois, is one of the major research centers of the North Central Region, Agricultural Research, Science and Education Administration (SEA-AR), U.S. Department of Agriculture. Basic and applied research is conducted in the physical and biological sciences and in engineering. Northern Center scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations, and with other Government agencies. Much of the cooperation is informal, but some work is conducted under cooperative agreements and memorandums of understanding. In addition, the Center's program is supplemented by a variety of research projects in foreign countries under Public Law 480 grants.

Providing scientific information for improvements in the post-harvest sector of American agriculture is a major mission of NRRC. Such improvements in the handling, storing, processing, and distribution technology through which agricultural products move "from farm gate to shopping basket" inevitably benefit both consumers and farmers. For example, research on this complex technology provides the basis for preserving and increasing food quantity, economy, quality, safety, and nutritive value. This research also offers our best hope for substantially reducing the energy intensiveness of post-harvest operations, which collectively require at least twice as much energy as is consumed on farms, and for innovations contributing to maintenance of environmental quality. Responding to these needs and opportunities, NRRC's research program and scientific staff provide SEA-AR's principal effort and expertise in processing and utilizing soybeans, cereal feed grains, and special crops. In addition, the Center is devoting attention to alternative energy resources from agriculture, through its involvement in the Northern Agricultural Energy Center.

Currently most of the soybean research is directed toward decreasing the cost and improving consumer acceptability and nutritional quality of the oil and of high-protein products derived from the meal. This research continues to justify the stature earned by past accomplishments which provided much of the fundamental basis for today's edible soybean oil and food grade soy protein industries. For instance, Center scientists pioneered in development of statistically reliable taste panel procedures that are essential for attaining improved flavor of soy-based foods. In the non-food area a nucleus still exists of a former sizable effort to exploit soybean and other vegetable oils as petrochemical-sparing industrial raw materials. From this nucleus the research could be expended rapidly, should national priorities so mandate.

Cereal grain research, on the other hand, is almost equally concerned with industrial and with feed and food uses. For example, Center scientists' special knowledge of the complex chemical and physical properties of corn starch and flour has enabled them to develop new technologies with promising on-farm applications. These include water pickup and retention by Super Slurper and controlled release of pesticides through encapsulation with derivatized starch. Recent research in the food area led to corn germ flour, and studies underway on food fiber are aimed at improving the healthfulness of the American diet. In addition, the Center staff continues to be a principal source of information and research on blended foods, such as CSM (from corn, soybean flour, and dry milk solids) for the Food for Peace program. Another major contribution to food science is research to determine the relationship between wheat proteins and their functional properties in bread and other baked goods. Ultimately these studies will provide fundamental insight relevant to nutritional products from other cereals as well as from oilseeds.

A key research resource, the Agricultural Research Culture Collection (NRRL), is a world renowned repository of agriculturally and industrially important microorganisms. Reference cultures, cataloged taxonomic data, and professional expertise associated with this microbial germ-plasm bank have enabled NRRC to make vital contributions and to assume preeminent roles in mycotoxin research and in fermentation technology, including production of food ingredients and fermented foods such as tempeh. Because of the unique capabilities of a multi-disciplinary staff and the importance of the problem, research on mycotoxins has become one of the largest components of the Center's overall effort. This research plays a key role in protecting our food supply from these hazardous substances.

Post-harvest processing behavior and product quality are markedly affected by preharvest factors. Recognizing this, Center chemists, engineers, microbiologists, and physicists participate in joint projects with other SEA-AR and SAES scientists conducting genetic and agronomic studies. Determination of processing and compositional characteristics of plant materials from botanical collections, breeding programs, and studies of soil and atmospheric variables is a major form of such participation. Another involves natural toxicants. Center scientists provide analytical and biochemical information necessary to make sure levels of these minor constituents are not seriously increased in new varieties. This work, like the mycotoxin research, helps assure the safety and nutritional quality of our food supply.

Center scientists have added their weight to the growing emphasis on photosynthesis, nitrogen fixation, and plant tissue culture. Their novel biochemical, microbiological, and physical approaches complement longer standing studies by plant physiologists and thereby expand and diversify the total SEA-AR attack in these high priority areas.

To take advantage of the resources and expertise available at NRRC, one of the two new SEA energy centers, the Northern Agricultural Energy Center (NAEC), is headquartered here. The goal of NAEC is to discover, develop, and

demonstrate technology which will permit agriculture to be energy self-sufficient on a net basis by 1990 under conditions that sustain productivity. The mission of the portion of the Energy Center program being carried out at NRRC is: (1) to develop innovative fermentative techniques emphasizing new microorganisms, new fermentation configurations, and use of substrates such as distressed grains; (2) to examine new approaches to substrate preparation and particularly investigate chemical, physical, and microbiological methods for conversion of cellulosic plant residues to sugars and thence to alcohol; (3) to evaluate the food and feed potential of nutrients isolated from fermentation residues and to develop methods to recover protein from these residues; (4) to expand the evaluation of plants as sources of hydrocarbons and to develop methods to separate and isolate hydrocarbon plant constituents.

This report summarizes research of the Center during calendar year 1979 and lists publications and patents resulting from the research. The research summaries include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are available to those having a special interest in the development of public agricultural research programs.

This report was prepared at the Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604. Additional copies of the report and reprints of most publications can be obtained from the Northern Regional Research Center. A separate annual listing of publications and patents also is available.

SELECTED ACCOMPLISHMENTS

Drying High-Moisture Corn. Granting of EPA clearance to permit use of gaseous ammonia as a mycostatic agent in grain drying gives the American farmer an inexpensive, energy-saving, quality-protecting means for preserving and drying high-moisture corn. [See Biomaterials Conversion Laboratory (BC), A.4.]

Encapsulation of Pesticides. The concept of using natural polymers as encapsulating agents for pesticides to improve efficiency of pest control is being advanced through cooperative efforts of AR scientists at Peoria, Beltsville, Purdue University, and University of Illinois. Improved controlled release of encapsulated pesticides has been realized through modifications of the natural polymer systems. Field tests with encapsulated herbicides show better weed control over a longer period of time than with standard commercial formulations. Indications are that controlled release formulations give better control with less pesticide. (See BC, B.5.)

Fuel Alcohol from Contaminated Grain. Ammonia-inactivation of aflatoxin-contaminated corn was integrated with the traditional ethanol fermentation process to yield ethanol for fuel and spent grain acceptable for feed use. The process provides an effective method for salvaging contaminated grain. (See BC, B.9.)

Wheat Gluten Structures Probed. By amino-terminal sequence analysis of alcohol-soluble gluten proteins, it was demonstrated that many subunits of glutenin, wheat's highest molecular weight protein class, are identical to an intermediate molecular weight fraction of gliadin proteins. This information shows how glutenin is formed, and how it interacts with other flour proteins to form gluten. Gluten expands and retains the gas produced during dough fermentation, giving bread a light desirable texture. These studies also show how differences in wheat quality may relate to key polypeptides; breeding for or modifying these polypeptides may improve quality. [See Cereal Science and Foods Laboratory (CSF), A.9.]

Cholesterol-Lowering Effect of Bran from Waldron Variety Wheat. A cooperative study with the SEA-AR Human Nutrition Research Laboratory, Grand Forks, North Dakota, has yielded a surprising result. A significant lowering of both total plasma cholesterol and low density lipoprotein cholesterol was observed when human volunteers ingested about 25 g per day of bran from Waldron variety wheat, a hard red spring wheat. (See CSF, D.1.)

Structure of an Unusual Rhizobial Polysaccharide. Structural characterizations were completed on the capsular polysaccharide (CPS) of the microsymbiont from nodules of *Acacia decurrens*. Methylation analyses of carboxyl-reduced and periodate-degraded CPS and of oligosaccharides from partial hydrolyzates revealed a complex structure. L-Rhamnose residues are the sole nonreducing end groups and are α -linked to three different sites in the CPS. Only one of

these residues becomes biologically 3-O-methylated as the culture ages. In Rhizobium japonicum CPS, progressive O-methylation of a nonreducing end group is thought to cover up the recognition site that binds with soybean lectin on root hairs. Characterization of oligosaccharide fragments of the CPS and methylation analyses revealed an underlying structure resembling that of R. japonicum CPS. [See Fermentation Laboratory (F), A.2.]

A New Medium for Differential Growth of Asymbiotic, Acetylene-Reducing Rhizobia. A new agar medium was developed for selection of rhizobia that can display nitrogenase activity in the free-living state. In addition, differential antibiotic sensitivities and nitrogen requirements were found to occur naturally among the rhizobia tested. These characteristics should be useful in physiological studies of the organisms and in selection of genetic recombinants with desirable traits; e.g., enhanced nitrogen-fixation efficiency and competitive surface recognition factors. (See F, A.3.)

Endotoxin Effects Upon Tissue Culture. Cultured insect tissue cells, when treated for 60 minutes with activated parasporal protein from B. thuringiensis, undergoes massive swelling of the cytoplasmic and nuclear membranes, followed eventually by complete lysis. Studies support the hypothesis that activated endotoxin acts as an ionophore upon gut tissue, thereby disrupting the normal distribution of cations on either side of the gut wall. (See F, B.1.)

Understanding the Species Concept at the Molecular Level. Standard physiological tests have proved inadequate for separation of yeast species, but now it is possible to define species on the basis of DNA relatedness. Strains are generally considered to belong to the same species when they exhibit 70-100% DNA relatedness, but they belong to different species when their DNA relatedness is only a few percent. Intermediate relatedness values (ca. 20-70%) are seldom found for yeasts. Recently, pairs of strains in three different genera were discovered which show 20-25% DNA relatedness. All pairs were capable of mating, but only one pair gave viable progeny. These data show that strains having only about 25% DNA homology may belong to the same species, and this provides important information at the molecular level for an understanding of the species concept. (See F, D.1.)

Reclassification of Actinomycetales Genera. The characteristics of some 50 genera in the bacterial Order Actinomycetales were restudied in light of advanced technologies that have come to the fore during the last decade and a new classification system was devised based on molecular biological, morphological, and biochemical bases. The new system provides a more objective basis for further study directed to elucidation of natural relationships of taxa within the Order, development of a geno-species concept, and more precise identifications. All this will enhance the use of strains of these microorganisms in a variety of fundamental and applied research studies. (See F, D.2.)

Culture Collection Rescues Important Collection of Standard Microorganism Strains. In June, the Collection accessioned some 3600 lyophilized preparations

of progeny of 605 Actinomycetales representing the original materials used for the International Streptomyces Project carried out from 1966-1972. These strains and their accompanying descriptions obtained from participants in the international cooperative study now are used worldwide in characterization and identification of streptomycetes and streptovercillia involved in patent applications and basic and applied research. Most of the methodologies involved were first developed in the Culture Collection. (See F, D.2.)

Immobilization of β -1,3 Glucanases. β -1,3 Glucanases from both Rhizopus arrhizus and a Cladosporium culture were successfully immobilized on activated granular charcoal. The hydrolytic action patterns on β -1,3 glucans displayed by the immobilized enzymes differed from the patterns obtained with the corresponding soluble enzymes. This finding is a significant development in our attempt to prepare derivatives of β -1,3 glucans that may have ability to cause plants to form elicitors necessary for their defense against certain fungal pathogens. (See F, D.3.)

Structural Characteristics of B-1355(S) Dextran. Heterogeneity in the structure of Leuconostoc mesenteroides NRRL B-1355 dextran fraction S was demonstrated by comparing the methylation analysis of the native dextran with that of a limit dextran prepared by extensive digestion with an exoisomaltodextranase. The limit dextran has almost twice the degree of branching and a lower amount of unbranched (1 \rightarrow 3)-linked residues than are found in the native polysaccharide. Furthermore, the limit dextran has about the same proportion of (1 \rightarrow 6)-linked residues as the native polymer. Consequently, the limit dextran must contain consecutive (1 \rightarrow 6)-linked glucosyl sequences located between the (1 \rightarrow 3)-coupled residues. This change in the ratio of (1 \rightarrow 6)-to (1 \rightarrow 3)-linked residues suggests that the structure of the polymer near the reducing end (limit dextran) differs from peripheral structure of the native dextran. Dextran B-1355 fraction S is of great interest in medically related fields as an agent for isolation of macroglobulins and in a variety of immunochemical, genetic, and other medical research. The dextran is the standard for assaying concanavalin A activity and is also used in research on this plant lectin. Consequently, any new information on its structure will be of wide interest to the scientific community. (See F, D.3.)

Pathogen Removal by Fermentation. Removal of potential microbial pathogens in the continuous fermentation of corn with cattle waste was demonstrated. Neither enteric nor acid-fast bacilli persisted beyond 6 hours. The numbers of viable bacteriophages (four strains) were reduced one-millionfold in 2 to 3 days; one, with lipid envelope, was killed within 5 minutes. Thus, the fermentation process kills bacterial pathogens that cause diseases such as salmonellosis, tuberculosis, or Johne's disease and severely limits the potential transmission of viral diseases. (See F, E.1.)

Lignin Decomposition. A fungus isolated from cattle dung effected substantial removal of lignin (45%) from wheat straw (WS) during fermentation at room temperature. The WS cellulosic remainder was digested to glucose six times more completely by enzymatic treatment than control straw. (See F, F.1.)

Innovative Guayule Rubber Extraction. A new method was developed for extracting rubber from guayule. Whole guayule plants were chopped and then subjected to compression and shear forces in a rubber mill that released the rubber for easy and efficient extraction with solvents. The process gave significantly improved extraction rates, decreased solvent holdup, and improved yields over prior methods of rubber extraction. (See F, F.1.)

Internal Bacterial Flora in Soybeans. In-situ heat-resistant bacterial spores of species of Bacillus in soybeans have interfered with processing of traditional soybean foods and have shortened the shelf-life of soybean foods. The microorganisms that are responsible for these difficulties have now been identified from an extensive survey of soybean samples of the 1978 crop from six different areas. The identification of the responsible microorganisms will facilitate the development of procedures to minimize the growth of these spore-forming bacteria during food processing. The bacterial contaminated rate ranged from 4 to 26%. As far as we know, no survey of the normal internal bacterial flora of soybeans has been made. (See F, G.1.)

Bioassay Developed for Detection of Feed Refusal Factors. Refusal of feed by swine has been a recurring problem in the North Central United States. Rejected corn generally contains vomitoxin, a metabolite produced by Fusarium graminearum and belonging to a class of compounds known as trichothecenes. These compounds are not readily identified by standard physical-chemical methods. To simplify analytical techniques, a mouse bioassay based on consumption of a mycotoxin in drinking water has been developed to determine the level of acceptance of 10 Fusarium metabolites. Because only the trichothecenes--diacetoxyscirpenol, T-2 toxin, and vomitoxin--were refused, the test should be useful in screening for these and related compounds. (See F, H.1.)

Analytical Method for Aflatoxins M₁ and B₁ in Liver. A method was developed for the determination of aflatoxins B₁ and M₁ in liver that can be used for regulatory purposes and research on transmission. The method is more reliable and convenient than those previously reported, and a rapid thin-layer chromatographic method for further confirmation of the identity of M₁ and B₁ in liver tissues has been developed. (See F, H.1.)

Fungal Colonists of Corn Interfere with Aflatoxin Contamination. Certain fungi commonly isolated from corn at harvest can affect aflatoxin development when inoculated with A. flavus onto individual sterilized corn kernels. No aflatoxins were detected when A. niger or Trichoderma viride were paired with A. flavus. The status of individual fungal colonists as interference competitors and the order in which species colonize a kernel (i.e., prior to, or simultaneous with, the introduction of A. flavus) must be examined as factors contributing to variation in aflatoxin levels among field samples. (See F, H.3.)

HPLC of Triglycerides. The analysis of seed oils, which are normally complex mixtures of triglycerides, was greatly advanced by the development of high-performance liquid chromatographic (HPLC) procedures for separating molecular species directly, without prior chemical modifications. Scientists at NRRC

have shown that triglycerides can be separated not only according to chain length, but also by functionality, such as double bonds, hydroxy, and epoxy groups. Correlations between the HPLC data and fatty acid compositions, as determined by classical gas chromatography, provided greatly increased insight into the relationship between triglyceride composition and chromatographic behavior. Determination of column specificity allows scientists to make critical decisions in choosing the appropriate column for particular separations. [See Horticultural and Special Crops Laboratory (HSC), B.2.]

Maytansinoids in Euphorbiaceae. A series of four new macrocyclic alkaloids, members of a group called maytansinoids, have been isolated from seeds of Trewia nudiflora; they are the first maytansinoids to be found in the plant family Euphorbiaceae. These new alkaloids have two kinds of biological activity which are potentially valuable: larvicidal activity towards the European corn borer and antitumor activity. (See HSC, B.3.)

Botanical Pest Control Agent. As part of a program of screening plants for pest control activity, extracts of Thevetia thevetioides (yellow oleander) seed (plant family Apocyanaceae) were found to be a potent larvicide towards the European corn borer. Fractionation and characterization have revealed that a toxic steroidal glycoside, neriifolin, is the constituent most lethal to these larvae; when added to the diet, it shows $LD_{50} = 30$ ppm. (See HSC, B.3.)

Selenium Protection Factors. For over 20 years, it has been recognized that flaxseed possesses unique properties in protecting livestock against the toxic effects of selenium compounds accumulated in forage plants. Two compounds responsible for this protection have been characterized--linustatin and neolinustatin. These compounds are previously unknown cyanogenic glycosides derived from β -gentiobiose. (Work carried out in collaboration with South Dakota State University, where the compounds were isolated; see HSC, B.3.)

Potato Slices for Antitumor Screening. Potato slices can be infected with crown gall tumor, the only true tumor known to affect plants. A variety of plant-derived antileukemic agents inhibit the development of tumors in these infected potato slices. Preliminary results indicate a positive correlation with activity in murine leukemia, but negative results with extracts that show only cytotoxicity by the conventional screening procedures. These observations suggest that this potato slice technique has the potential for greatly facilitating screening of plant extracts for antitumor activity because of its speed, low cost, and low sample requirement. (Work carried out in collaboration with Bradley University; see HSC, B.3.)

Biological Effects of Glucosinolates. Nine aglucon products from glucosinolates found in cruciferous vegetables have been evaluated as teratogens in the rat. While none were teratogenic, three caused decreases in fetal weight: 1-cyano-3,4-epithiobutane, allyl isothiocyanate, and 3-methylsulfinylpropyl isothiocyanate. 1-Cyano-2-hydroxy-3-butene caused rolling seizures in pregnant rats comparable to neurolathyrism; 3-indolylacetonitrile caused sedation and ataxia in pregnant rats. (See HSC, G.2.)

Photosynthesis and Heat Stress in Plants. The serendipitous observation that brown sea kelp turns green on heating to 55°C may find practical implications for crop plants under heat and water stress. More important than the color change is the disruption of light energy transfer. The orange pigment of the diatom no longer contributes its absorbed light to green chlorophyll and to photosynthesis of the cell. Similarly, in the green algal species Chlorella pyrenoidosa, which serves as a model for crop plants, the light energy absorbed by chlorophyll b is no longer transferred to chlorophyll a and to photosynthesis. [See Oilseed Crops Laboratory (OC), A.1.]

Light Harvesting Caroteno-Chlorophyll-Protein Complex Isolated. By use of exceptionally mild conditions, an orange-colored caroteno-chlorophyll protein has been isolated from the diatom Phaeodactylum tricornutum. The efficiency of light energy transfer from carotenoid pigments to chlorophyll a is nearly equivalent to that of the intact algal cell, but it is destroyed by sonication and detergents normally used in protein isolations. Molar proportions of carotene, chlorophyll a, chlorophyll c, fucoxanthin, and neofucoxanthin are 1:3:3:12:4. Light-harvesting caroteno-proteins also occur in higher plants and may, therefore, also contribute to increased photosynthetic efficiency and yield of the crop plants through increased absorption of light. (See OC, A.1.)

Photosynthetic Process Computer Simulated. One of the paradoxes of photosynthesis has been that the plant can split water to give off oxygen, a process requiring 112 kilo calories, in red light where the quantum of light energy is only 30 kilo calories or one quarter that required. It is known from experiments involving high-intensity flashes of light that four quanta are stored and oxygen is evolved on every fourth flash. Now it has been shown that this cycle of four curve for oxygen evolution progressively degenerates to a simple "S" curve when the intensity of light flashes is reduced 100 fold, and this behavior has been computer simulated using target theory of "hits" and "misses" and the binomial theorem. (See OC, A.1.)

Model Light Capture System. A system has been devised consisting of chlorophyll a and chlorophyll b adsorbed on a specific hydrophobic surface. Chlorophyll a and chlorophyll b adsorbed together show individual and additive light absorption characteristics but, under certain conditions, only the red fluorescence of chlorophyll a. This means, as in the intact green leaf, light energy absorbed by chlorophyll b is transferred to chlorophyll a, causing chlorophyll a only to emit fluorescent light. This pioneering experiment opens a new path for understanding how light energy is absorbed, transferred, and used in photosynthesis. (See OC, A.2.)

Tasting Procedures Guide Soy Protein Purchases by Defense Department. Taste-panel procedures developed at NRRC for evaluation of soy protein products are considered to be the standard of the industry. In 1979, they were adopted by the U.S. Army Natick Research and Development Center as part of their specifications for purchase of textured soy proteins to be used in preparation of

beef-soy blends. The Department of Defense is testing the use of beef extended with 20% soy protein and has found that use of soy causes no loss in nutritional value. Adoption of beef-soy blends by the military was projected to result in savings of over \$6 million annually. (See OC, B.1. and B.8.)

Deterioration of Vegetable Oils by Light. Traces of natural photosensitizers in soybean oil and other vegetable oils were shown to catalyze the oxidation in light of linoleate, the main polyunsaturated fatty acid constituent. This deterioration by light was shown to proceed by an entirely different mechanism than air oxidation and requires different control measures. The light mechanism involves "singlet" oxygen, an activated form of oxygen that can be inactivated by naturally occurring quenchers such as β -carotene and α -tocopherol. This basic information is required to develop better methods of control in light oxidation. (See OC, B.6.)

Quality of Steam-Refined Soybean Oil. Sensory evaluation of laboratory steam-refined soybean salad oils indicate flavor stability during storage comparable to that of conventionally refined oils. Investigations demonstrated that pretreatment of the crude oil with phosphoric acid is critical to producing quality steam-refined oils. These findings have served to stimulate considerable interest in applications of steam refining to preparation of finished soybean salad oil. (See OC, D.1.)

Simultaneous Deacidification-Deodorization. Steam refining of edible oil has been proposed as a means of minimizing pollution-control and waste-disposal problems inherent with the common alkali refining technique. Additional advantages of energy conservation and cost reduction are anticipated. (See OC, D.1.)

Utilization of cis- and trans-12-Octadecenoic Acid Isomers in Man. A detailed study of the incorporation of the cis- and trans-12-octadecenoic acid isomers into human blood lipids has been completed and represents the only human experiment available for assessing the relative nutritional value of these fatty acid isomers, which are formed during hydrogenation of vegetable oils. Data indicate the 12-octadecenoic acid isomers are biologically and nutritionally significant. The primary reason for the cis-12-octadecenoic acid's importance is its ability to compete with the essential fatty acid, linoleic acid, for incorporation into phospholipids. (See OC, E.1.)

Synthesis of Polyunsaturated Fatty Acid Isomers. The biological properties of the linoleic acid are of considerable importance. However, no information based on human experiments is available concerning the biological properties of the 12,15-octadecadienoic acid isomers of linoleic acid which are formed during hydrogenation of vegetable oils. This isomer is difficult to prepare as a pure compound because of the formation of four geometrical isomers. Synthesis of all four of these isomers has been accomplished based on a technique that controls the stereochemistry of the Wittig reaction. In addition, the synthetic scheme incorporates two deuterium tags, which allows the metabolism of these isomers in humans to be studied. (See OC, E.1.)

BIOMATERIALS CONVERSION LABORATORY

W. M. Doane, Acting Chief

Research Leaders: R. A. Anderson, M. O. Bagby, R. J. Bothast,
G. E. Hamerstrand, G. C. Mustakas, and F. H. Otey

A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Engineering Factors Affecting Design and Operation of Immobilized Enzyme Processes for Foods and Feeds (E. B. Bagley)

- a. Specific Objectives: To refine and extend both the experimental data base and the mathematical model for the enzyme conversion of raffinose and other oligosaccharides to component monosaccharide.

Progress: Cooperative work with SEA's Data Applications System Division and Control Data Corporation on the mathematical modeling of the operation of a continuous hollow fiber enzyme reactor was continued. Initial work was based on a simple, one-enzyme system following Michaelis-Menton kinetics. The model fits well not only for the expected systems (sucrose-invertase) but for more complex systems (e.g., raffinose-alphagalactosidase-invertase). Batch studies indicated that previously unrecognized product inhibitions were occurring.

Publications:

MCGHEE, J. E., SILMAN, R. W., AND BAGLEY, E. B. Production of alpha-Galactosidase from Aspergillus awamori: Properties and Action of Para-nitrophenyl alpha-D-Galactopyranoside and Galactooligosaccharides of Soy Milk. J. Am. Oil Chem. Soc. 55 (1978):244-247.

SILMAN, R. W., BLACK, L. T., MCGHEE, J. E., AND BAGLEY, E. B. Hydrolysis of Raffinose in a Hollow-fiber Reactor Using an Unrefined Mixture of alpha-Galactosidase and Invertase. Biotechnol. Bioeng. In press.

SILMAN, R. W. Enzyme Formation During Solid Substrate Fermentation in Rotating Vessels. Biotechnol. Bioeng. 22 (1979):411-420.

2. Basic Engineering Studies on Preparation of Soy Foods (G. C. Mustakas)

- a. Specific Objective: Evaluate methods for investigating the viscoelastic properties of soy-based doughs and relate these measurements to composition, specific interactions, and to the effects of subsequent processing on final properties.

Progress: Methodology for investigating the rheology of soy doughs using a mechanical spectrometer in ERD (Eccentric Rotating Disk) mode was developed. It was found that moisture loss from the sample surface had to be controlled before useful data on the viscoelasticity of soy dough could be collected. Effects due to machine variables such as plate diameter, sample thickness, and angular velocity were determined. Effects due to sample variable such as dough moisture nitrogen solubility index and percent strain were also investigated.

- b. Specific Objective: Investigate basic engineering and rheological aspects of extrusion in relation to soy quality in foods.

Progress: An extruder with capillary dies of a variety of dimensions was used to investigate the rheological properties of defatted soy dough. Theories developed for extrusion of thermoplastics were used to evaluate data collected at various shear rates and die sizes. Information on the rheological properties (viscosity and elasticity) of soy doughs was analyzed. It was found that residence time in the extruder affects the viscoelastic properties of the extrudate, especially its elastic component.

- c. Specific Objective: Establish relationship between soybean processing variables and the nutritive value of soybean meal in the diet of the chick (cooperative research with University of Maryland Animal Science Department).

Progress: A total of forty-eight test runs were completed in the extraction pilot plant in which 25 pounds of defatted toasted meal were prepared from each run and shipped to the University of Maryland for poultry feeding. An integral part of the plant was the experimental toaster which not only was used to simulate the commercial toasting process but also was effective in evaluating critical parameters in the process.

- d. Specific Objective: Investigate the preparation of purified trypsin inhibitors (TI) from a variety of food sources using ultrafiltration and other techniques to study the biochemical effects in long term rat feeding studies (cooperation with Washington State University, WRRRC, Albany, CA, and ERRC, Wyndmoor, PA).

Progress: Potatoes are a rich source of TI. During the latter part of the year, ERRC processed 12 tons of potatoes to produce 1300 lb of potato juice concentrate which was frozen and shipped to NRRC. Preliminary experiments are now underway to study protein recovery and TI concentration by ultrafiltration techniques. Approximately 25 lb of purified TI will be prepared to determine its effect on the pancreas of rats fed varying levels of casein.

- e. Specific Objective: Investigate alternate approaches to the hexane solvent extraction procedures for soybeans (cooperation with Shell Development Corporation).

Progress: The soybean extraction pilot plant at NRRC is being modified to enable extraction with aqueous alcohols rather than hexane. These modifications are being made to accommodate anticipated changes for such variables as extraction temperature, solvent-to-flake ratio, solvent recycling and methods of solvent foil separation. A memorandum of understanding for a cooperative program with Shell Development Corporation, Houston, Texas, has been prepared and is being negotiated.

- f. Specific Objective: Investigate the bioavailability of zinc and other minerals in soy protein diets (cooperative project with the University of Illinois Food Science Department).

Progress: Soy products with low, intermediate and normal phytate levels were prepared in the pilot plant for subsequent rat feeding experiments at the University of Illinois to evaluate zinc bioavailability. The low and intermediate level phytate products were made by precipitation of the protein curd at pH 5.5 and 4.5 respectively. The higher level phytate product was produced by a similar process except that the phytate which had been previously isolated from the whey was recycled back to the original process as native phytate.

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Reports:

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JASBERG, B. K., N. W. TAYLOR, G. C. MUSTAKAS, AND E. B. BAGLEY. Determination of Dynamic Moduli of Soy Doughs Using an Orthogonal Rheometer. Presented at the 50th Society of Rheology Meeting, Boston, Massachusetts, October 28-November 2, 1979.

MOULTON, K. J., G. C. MUSTAKAS, AND E. C. BAKER. Pilot-Plant Desolventizing-Toasting of Extracted Soybean Flakes-A Preliminary Batch Study. Presented by G. C. Mustakas at International Society for Fat Research and American Oil Chemists Society, New York City, New York, April 27-May 1, 1980.

MUSTAKAS, G. C. Recovery of Oil from Soybeans. AOCs Short Course, Lake Kiamesha, New York, April 20-23, 1980.

MUSTAKAS, G. C., E. D. MILLIGAN, J. A. LABORGA, AND D. A. FELLERS. Conversion of Soybean Extraction Plant in Bolivia to Production of Flours for Human Consumption. Presented at the 70th Annual Meeting, AOCs, San Francisco, California, April 25-May 3, 1979.

3. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (R. A. Anderson)

- a. Specific Objective: Reduce energy requirements in the corn milling industry.

Progress: Dry milling studies on high-moisture corn from the 1978 crop, which had been dried with ambient air while using sulfur dioxide as a preservative, indicated that lower yields of prime products (grits, low-fat meal and flour) were obtained as compared to air-dried control corn or corn dried by the trickle ammonia process. However, fat content of the fractions from the SO₂ treated corn were similar to control.

Yields of fractions from the dry milling of high-oil corn (8.7% fat) were similar to those obtained from the milling of a normal dent corn (4% fat). But the quality of the grits and other low-fat fractions was decreased, as the fat content of these fractions was 1.5 to 7 times greater than normally obtained from regular corn. This appears largely due to the expelling of oil and the coating of endosperm particles during the roller milling operations.

Bench-scale procedures for the dry and wet milling of corn have been standardized to provide methodology for studying interphased processing of this grain.

- b. Specific Objective: Corn germ flour from interphase milling.

Progress: Methods of preparing corn germ flour from interphase milling germ were investigated to reduce energy and improve flavor stability of food-grade flours. Bran and endosperm milling contaminants can be separated from meals ground in hexane by direct wet screening or by differential density fractionation during oil recovery. However, grinding disrupts particulates of the germ and smears hydrophobic phospholipids and free fatty acids on the meal particles. This improved availability of rancidifying constituents increase off-flavors during storage. Partial emulsification of phospholipids and hydration of pectins and

pentosans with a short water temper of the whole fat germ produces a gummy fraction which can be removed with the feed fraction. Almost 60% of the phospholipids can be removed from the food-grade protein flour.

Reports:

ANDERSON, R. A. Current Status of Research and Development in the Dry Milling Industry. Presented at the Workshop on Processing, Utilization and Marketing of Maize, New Delhi, India, March 19-22, 1979.

PEPLINSKI, A. J., R. A. ANDERSON, AND O. L. BREKKE. Corn Dry Milling: Influence of Harvesting and Drying. Presented at the Annual Meeting of the American Society of Agricultural Engineers, New Orleans, Louisiana, December 11-14, 1979.

4. Development of Mycostatic Systems to Permit Safe, Low-Energy Grain Drying (R. A. Anderson)

- a. Specific Objective: Explore ambient air drying of high-moisture corn using extenders such as ammonia and sulfur dioxide in small and largescale tests as well as develop Environmental Protection Agency approval for the process.

Progress: Environmental Protection Agency approval for the "Trickle Ammonia Process" (TAP) for preserving and ambient air drying of high-moisture corn was received July 31, 1979 (44 FR 44844). In field tests using high-moisture corn from the 1978 crop year, 1,500 bushels of corn was dried from 26.8% to 14.5% moisture in 44 days. During this period, 114.8 lb of ammonia gas and 1,129 kwh of electricity were used. TAP drying cost totalled 4.7 cents a bushel, as compared to a calculated cost of 10.6 cents per bushel for high-temperature drying.

In 1978 field tests, sulfur dioxide (SO₂) was an effective mycostat during low temperature drying of high-moisture corn. Intermittent application of 0.14% SO₂ to 365 bushels of 26% moisture corn permitted low flow ambient air drying over 66 days. Internal kernel molds were reduced from 96% to 4% and bacteria and molds on the kernel surface were effectively controlled. The treated corn satisfactorily met all quality criteria according to the Official United States Standards for Grain. A petition has been submitted to the Environmental Protection Agency for clearance to use sulfur dioxide as a mycostat in the "trickle SO₂" process.

Publications:

BOTHAST, R. J. AND R. A. ANDERSON. Trickle Ammonia Process-- Preservation of Corn While Drying with Ambient Air. CA-NRRC-45, December 1979.

MCGHEE, J. E., E. B. BAGLEY, AND R. J. BOTHAST. Treating Corn with Aqua Ammonia: Effect on Meal Constituents. Cereal Chem. 56 (1979):128-130.

NOFSINGER, G. W., R. J. BOTHAST, AND R. A. ANDERSON. Field Trials Using Extenders for Ambient-Conditioning High-Moisture Corn. Trans. ASAE 22 (1979):1208-1213.

B. TECHNOLOGIES FOR INDUSTRIAL USES OF PLANT AND ANIMAL PRODUCTS

1. Nonpollutant Paper and Textile Additives from Cereal Flours and Starches (W. M. Doane)

- a. Specific Objective: Finish studies in progress on synthesis and chemical characterization of quaternary amine-starch derivatives made by dry-state grafting of starches with acrylic and methyl acrylic esters and amides.

Progress: The new procedures through dry blending of reactants drastically reduce energy requirement/unit by (1) realization of room temperature reactions achieved over several days and (2) elimination of drying requirement or solvent removal and disposal. Additionally, granule swelling inhibitors are not necessary, thereby providing further cost reduction. The basic process is a radical departure from conventional preparative methods; the driving force results from a 10-20 fold increase of effective reactant concentration yet conditions lead to homogeneous substitution of reactants.

- b. Specific Objective: Study of the reaction of carbamoylethyl starch and sodium hypochlorite to minimize hydrolysis of isocyanate intermediates and increase crosslinking (isocyanate-active hydrogen).

Progress: A study of reactions of carbamoylethyl starch (CES) with sodium hypochlorite to minimize hydrolysis of the isocyanate intermediate and to increase crosslinking of the chlorinated CES derivatives in situ as sizing agents for paper has been essentially completed.

Variables investigated include molecular weight of starch substrate, degree of substitution, alkali/hypochlorite/amide substituent mole ratios, pH, time, temperature, and numerous variables involving sizing applications. These parameters have been optimized on a laboratory-scale basis resulting in (1) improved stability of the reactive species prior to application, (2) highly improved paper wet- and dry-strength properties, (3) unusual degrees of improved starch retention in the sized paper, and (4) a recommended procedure for potential industrial application.

Publications:

CARR, M. E. AND B. T. HOFREITER. Ampholytic Starch Wet-End Additives. *Staerke* 31(4) (1979):115-120.

CARR, M. E., R. G. FECHT, AND B. T. HOFREITER. Chlorinated Carbamoyl ethyl Starch for Wet-Rub Resistant Paper Coatings. *Tappi*, in press.

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HAMERSTRAND, G. E., H. D. HEATH, B. S. PHILLIPS, J. C. RANKIN, AND M. I. SCHULTE. Cationic Surface Sizes - Impact on Secondary-Fiber Furnishes. *Tappi* 62(7) (1979):35-38.

RANKIN, J. C. The Nonfood Uses of Corn. Accepted for publication in *CRC Handbook of Agriculture--Processing and Utilization*.

2. Starch-Based Copolymers for Making Elastomers (T. P. Abbott)

- a. Specific Objective: Make an ionic starch by graft polymerizing monomers to produce low molecular weight side chains which may be converted to ionic derivatives of starch for use as an ionic filler in rubber.

Progress: Crotyl acrylate was chosen as best from several monomer candidates studied for oligomeric grafting. Grafts were initiated with 0.25 moles Ce(IV) per mole starch anhydroglucose unit, a higher than usual amount of initiator, to increase the frequency of grafting sites while maintaining a low monomer to starch ratio. High conversion of monomer to polymer was obtained. More crotyl

acrylate was insolubilized in the presence of starch than in the monomer alone when irradiated with less than 0.25 Mrad of cobalt-60 gamma rays. Initiation by starch is therefore evident and probably grafting onto starch takes place. More than 50% of the starch-crotyl acrylate is insoluble in DMSO or 1N NaOH, even at low levels of crotyl acrylate grafting. Apparently, the crotyl acrylate acts as a crosslinking agent for starch as in the Ce(IV) initiated samples.

- b. Specific Objective: Determine the parameters which cause variation in the ratio of aromatic to aliphatic proton resonance peak areas in the NMR spectra of poly(styrene sulfonic acid, sodium salt).

Progress: Concentration of the polymer, presence of excess NaCl, and deuterium exchange by D₂O appear to have no effect on the NMR spectrum of poly(styrene sulfonic acid, sodium salt) but pH of the polymer or percentage of sulfonic acid moiety neutralized does change the ratio of aromatic to aliphatic proton resonance peak areas.

3. Starch-Based Purification Aids for Wastewater (W. M. Doane)

- a. Specific Objective: Determine the effectiveness of starch-based products for cadmium removal from wastewaters.

Progress: Insoluble starch xanthate (ISX) and iminodiacetic acid crosslinked starch (IDACS) were evaluated on cadmium containing solutions at pH's less than 9. These starch-based products were very effective in binding and removing cadmium. Neutralization of cadmium containing rinsewaters with caustic or lime was effective in precipitation of cadmium hydroxide but only at pH's greater than 10. ISX and IDACS are cost effective cadmium scavengers at low initial cadmium concentrations (< 10 mg/l). Caustic is cost effective on more concentrated cadmium containing wastewaters.

- b. Specific Objective: Evaluate actual cadmium laden rinsewaters with starch-based products.

Progress: Several acid cadmium plating rinses were obtained from plating suppliers. Insoluble starch xanthate and iminodiacetic acid crosslinked starch were used to treat various cadmium concentrations in the wastewaters. Residual cadmium concentrations were well below Environmental Protection Agency limits. The cadmium-starch-based product sludges were very stable and were found suitable for discharge to approved landfills. Cadmium recovery from starch-based sludges was accomplished by treatment with 4N nitric acid or incineration.

Publications:

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4. Flame-Resistant Polyurethane Foams and Biodegradable Films and Packaging Prepared from Starch (F. H. Otey)

- a. Specific Objective: Determine effect of molecular structure and formulation on rate of fungi and UV degradation of biopolymer-based films and plastics that have potential application as biodegradable agricultural mulch, planters, and chemical containers.

Progress: Some preliminary biodegradation tests were conducted by exposing starch-based film samples to outdoor and indoor soil contact; however, more controlled data were obtained by placing test specimens on solid agar growth medium and inoculating them with test microorganisms commonly found in the soil according to ASTM D 1924-70. The amount of mold coverage on each specimen was recorded each week for 4 weeks. With 10 to 30% starch and 70 to 90% ethylene acrylic acid copolymer (EAA) films, no mold developed due to fungi attack, and with 40% starch and 60% EAA only 10 to 20% of the specimen was covered with mold. At 50% and higher starch levels, the films were readily attacked indicating that the samples would biodegrade within a few weeks upon exposure

to outdoor soil contact. When low density polyethylene (LDPE) was incorporated into the starch EAA composition, fungi attack occurred readily even with 10 to 20% starch present.

UV degradation studies were conducted in a weatherometer and reported as failing when small cracks developed in the film. With 10% starch the films remained in good condition for 400 hours. Resistance to UV exposure decreased with increasing starch levels. Those with 40 and 50% starch lasted only 90 hours. Addition of LDPE greatly improved UV stability such that films with 40% starch, 10% EAA and 50% LDPE lasted 560 hours before cracks developed.

- b. Specific Objective: Continue to study the interaction between biopolymers and synthetic polymers to establish preferred systems for making biodegradable films and plastics.

Progress: Various combinations of biopolymers and synthetic polymers were formulated and evaluated for flow and compatibility by using extrusion blowing equipment. In earlier studies we demonstrated that quality films could be cast from aqueous dispersion of starch and EAA; however, these compositions did not have the desired flow and compatibility characteristics for producing films with the more economical extrusion blowing equipment. Quality blown films were obtained by adding ammonia to a starch-EAA formulation and by controlling the moisture content just prior to extrusion at 5-8%. The ammonia apparently improved the dispersibility of EAA in water and reduced the rate of starch retrogradation both of which would improve compatibility between starch and EAA molecules. Evidence of ammonia in the final product was shown by an IR peak at 1550 cm^{-1} wavenumber which is in the range of a carboxylate group. The new system also allowed the incorporation of low density polyethylene as a partial replacement for the EAA which greatly reduced cost and improved some properties of the film.

- c. Specific Objective: Continue a systematic approach to find an optimum method for plasticizing starch film with the ultimate aim of establishing a correlation between the chemical structure of polyols and other classes of organic compounds and their effectiveness as plasticizers for starch films.

Progress: The synthesis of 1,2,3-butane triol, 1,2,3-pentane triol, 1,2,4-pentane triol, 1,2,5-pentane triol, and 2,3,4-pentane triol, each from an appropriate unsaturated aliphatic alcohol by way of the addition hydroxylation-formesterification reaction of $\text{H}_2\text{O}_2 + \text{HCOOH}$, was started. These polyols are being prepared to establish a correlation between chemical structure and effectiveness as film plasticizers.

Films have been prepared from the reaction products (in a series) of reactions in which reaction parameters were varied of alkali-gelatinized starch and a (poly) methyl vinyl ether: maleic anhydride, and alkali-gelatinized starch and benzophenone tetracarboxylic acid dianhydride. The two reagents provide essentially equal molecular weights, 156 and 161 respectively, in a crosslinking entity per anhydroglucose unit, molecular weight of 162, but quite different structural characteristics. Films prepared from both reaction products were plasticized with glycerol at 18-20% by weight of total film solids, cast from water. It was found that the (poly) methyl vinyl ether: maleic anhydride adduct produced the best film strengths when present at about one degree of substitution on the starch, that is, there was a 1:1 mol relationship between reactant and starch. On the other hand, the dianhydride reactant produced best film strengths at a 0.1, or perhaps less, degree of substitution; but there are indications that at higher ratios there may be possibilities for a stronger plastic development. Both reaction products should project a biodegradable nature as an ester link is formed in the union to the starch, and a carboxyl group formation accompanies on the appendant part of the crosslinking entity.

- d. Specific Objective: Expand cooperative efforts with AR soil and plant scientists to determine the feasibility of using biopolymer-based films for agricultural applications.

Progress: Techniques have been developed for formulating high levels of starch, and various levels of ethylene acrylic acid copolymer and polyethylene into compatible systems that can be blown into biodegradable film. The films have potential application in agricultural mulch, packaging, and planters. Small samples were sent to AR scientists but evaluation results have not been received.

Publications:

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MAHER, G. G. Crosslinking of Starch Xanthate. V. Redox Grafting with Hydrogen Peroxide and Vinyl Monomers in Water. Polym. J. (Japan) 11 (1979):85-94.

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5. Controlled Release of Pesticides by Formulating With Cereal-Based Starch and Flour Derivatives (W. M. Doane)

- a. Specific Objective: Study variables in preparation of cellulose and starch encapsulated pesticides to optimize performance of experimental formulations.

Progress: Variables in preparation of cellulose and especially starch encapsulated pesticides were studied. As a result a new method was discovered that eliminates the need of xanthate group for encapsulation and hence the need of use of CS₂ which is toxic and very flammable. The new procedure is based on precipitation of starch in the presence of core material such as a calcium adduct. Herbicides, insecticides, and nematocides were successfully encapsulated by the new procedure.

- b. Specific Objective: Elucidate the mechanism of release of active agents from starch or cellulose encapsulated formulations.

Progress: Examinations by scanning electron microscopy of encapsulated pesticides made by the xanthate or calcium adduct procedures of several classes of pesticides revealed that the surface of the granules are continuous and fairly smooth on the outside but a sponge-like structure containing microcells of the entrapped pesticide on the inside. These studies provided the first step in understanding the mechanism of encapsulation.

- c. Specific Objective: Continue to cooperate with AR, industrial, and university scientists on evaluation of the starch-based slow release herbicides, insecticides, and nematocides.

Progress: Cooperation with many SEA-AR weed scientists, entomologists, and nematologists, and with scientists at universities and industry continued. NRRC chemists prepared numerous starch encapsulated pesticides, made via starch xanthate, for greenhouse and field testing by the cooperating scientists. A major effort was concentrated on formulating the insecticide-nematicide Diazinon and the herbicide Treflan. New formulations of both pesticides were made and tests showed improved storage stability of Diazinon and improved efficiency of Treflan. The rate of release of Treflan from encapsulated and adsorbed formulations with starch xanthide were determined in the dry state and in the presence of water.

Four starch-bonded herbicides (a. picloram-pregelatinized starch, b. picloram-unmodified starch, c. 2,4-D-pregelatinized starch, and d. 2,4-D-unmodified starch) were tested in sterilized and unsterilized soil using lettuce as the target plant. Non-sterile soil showed good herbicide release (no lettuce growth). The rate of release was $a > b > c \gg d$. Sterile soil showed no herbicide release unless small amounts of non-sterile soil were mixed in the media solution of the starch-herbicide before addition to the test pots. Formulations containing mixtures of starch-herbicides were effective in varying the release rate with time.

Publication:

STOUT, E. I., B. S. SHASHA, AND W. M. DOANE. Pilot-Plant Process for Starch Xanthide Encapsulated Pesticides. J. Appl. Polym. Sci. 24 (1979):153-159.

6. Graft Polymers of Starch for Agricultural Chemicals and Absorbents
(G. F. Fanta)

- a. Specific Objective: To better understand the mechanism of water swelling of hydrolyzed starch graft copolymers and other gels.

Progress: Starch-polyacrylonitrile (PAN) graft copolymers were prepared via ceric ammonium nitrate initiation from both granular starch and from starch which had been gelatinized by heating in water at 85°C. To gain some insight into possible crosslinking mechanisms, a wide range of starch AGU:ceric ion molar ratios was used, and graft copolymers were characterized as to weight % add-on and the molecular weight of grafted PAN. Graft copolymers were then saponified by heating in aqueous alkali, and the water absorbencies and water solubilities of saponified starch-g-PAN copolymers were then determined. With granular starch, ceric ion concentrations ranging from 1 mole per 10 AGU to 1 mole per 500 AGU gave about the same % add-on (50%). There was little effect on graft molecular weight in the 50-200 AGU per ceric ion range. With gelatinized starch, % add-on was nearly constant at 50-200 AGU per ceric ion, although graft molecular weights were appreciably higher than those obtained with granular starch. Gelatinized starch yielded saponified starch-g-PAN copolymers with higher water absorbencies than the corresponding granular starch-based products; however, within certain limits, absorbencies were not overly sensitive to the amount of ceric ion used. Graft polymerization onto waxy corn starch, high amylose corn starch, and acid modified corn starch was also studied as well as the properties of the corresponding saponified polymers. These starches gave higher absorbency products than normal corn starch, with the exception of gelatinized high amylose starch, which

afforded a much lower absorbency polymer. Portionwise addition of ceric ammonium nitrate led to higher absorbency products, as compared with addition of the initiator in a single portion.

- b. Specific Objective: Study the graft polymerization of high molecular weight acrylates and methacrylates onto carbohydrates.

Progress: A series of C₄-C₁₂ alkyl acrylates and methacrylates was polymerized with starch by irradiating starch-monomer mixtures with cobalt-60. Homopolymers were extracted with cyclohexane. The amounts of insoluble vs. soluble synthetic polymer in polymerizations run with alkyl acrylates varied less with the chain length of the alkyl substituent than in polymerizations run with alkyl methacrylates, and poly(alkyl acrylate) contents of cyclohexane-insoluble fractions were all in the 38-45% range. Synthetic polymer contents of the products from butyl, hexyl, and decyl methacrylates were also close to this range. Octyl and lauryl methacrylate, however, gave high conversions to cyclohexane-soluble poly(alkyl methacrylate) along with little or no unextractable synthetic polymer in the starch-containing fractions. Poly(lauryl methacrylate) could be rendered insoluble by incorporating a small amount of tetramethylene glycol dimethacrylate in the polymerization mixture. In a series of polymerizations run with hexyl acrylate and hexyl methacrylate, lower irradiation doses led to more cyclohexane-soluble polymer and less synthetic polymer in the starch-containing fractions. Enzymatic digestion of starch-containing polymers gave synthetic polymer fractions which were largely insoluble in cyclohexane. Crosslinking is, therefore, probably taking place during these polymerizations; however, we could not eliminate the possibility that reduced solubility was caused by small amounts of residual carbohydrate in these polymer fractions. Ceric ammonium nitrate-initiated polymerizations of butyl acrylate, hexyl acrylate, and butyl methacrylate with starch gave cyclohexane-insoluble polymers which contained 33-39% synthetic polymer. The higher alkyl acrylates and methacrylates produced little or no polymer under these conditions. Starch-containing fractions were tested as absorbents for hydrocarbons. Products prepared from decyl acrylate and lauryl acrylate absorbed about 9 g of isooctane per g of polymer, while the lower alkyl monomers gave polymers with lower absorbency.

- c. Specific Objective: Determine the effect of reaction temperature on graft molecular weight when poly(methyl acrylate) is graft polymerized onto starch and other carbohydrates; also study the esterification of the carbohydrate components of these graft copolymers.

Progress: Removal of starch by perchloric acid oxidation from starch-poly(methyl acrylate) (PMA) graft copolymers gave PMA samples which were not completely soluble in acetone. Graft copolymers prepared at 25°C typically yielded PMA which contained about 1-5% by weight of insoluble gel. PMA isolated from a graft copolymer prepared at 50°C contained 15% insolubles. Other methods of starch removal (enzyme or refluxing hydrochloric acid) yielded polymers with larger gel fractions. Starch-poly(methyl acrylate) graft copolymers could be acetylated to various degrees of substitution (D.S.) with acetic anhydride in pyridine. Acetylating to a D.S. of 0.62 did not improve extrudability.

- d. Specific Objective: Examine the extrusion of carbohydrate-poly(methyl acrylate) graft copolymers and study the physical properties of the resulting plastics.

Progress: If starch-poly(methyl acrylate) graft copolymer was masticated between the cold rolls of a rubber mill prior to extrusion, a more flowable polymer was obtained which gave a tougher and stronger extruded plastic than that obtained from the unmasticated control (e.g., UTS = 2,970 psi vs. 2,100 psi for the control). Moreover, mastication gave a polymer which could be blow molded to a film.

Publications:

TAYLOR, N. W. Rheology and Water Swelling of Carboxymethyl Starch Gels. J. Appl. Polym. Sci. 24(9):2031-2040. November 1979.

TAYLOR, N. W. AND E. B. BAGLEY. Stress Yield Value in Closely Packed Gel Suspensions. J. Appl. Polym. Sci. 23(6):1897-1900. March 1979.

DOANE, WILLIAM M., Commercialization of a New Starch-Based Polymer. ACS Symp. Ser. 105 (1979):161-163.

Reports:

PRYDE, E. H. Fatty Acid Derivatives and Their Possible Uses. Presented before the Research and Technical Committee of the Fatty Acid Producer's Council, Northern Regional Research Center, Peoria, Illinois, May 22, 1979.

PRYDE, E. H. Recent Research on Nonfood Uses for Soybean Oil. Presented at the Conference of General Collaborators, North Central States, at the Northern Regional Research Center, Peoria, Illinois, October 22-23, 1979.

Several popular articles appeared in newspaper, trade journals, and magazines on Super Slurper, the highly absorbant starch-based polymer.

7. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. O. Bagby)

- a. Specific Objective: Identify U.S. plant species that give promise as potential multi-use hydrocarbon producing crops.

Progress: Several thousand samples initially screened as sources of potential biologically active agents were obtained from Raltech, Madison, Wisconsin. About sixty new species were collected mainly from the mid-west. The leguminoseae family was emphasized.

Publications:

BUCHANAN, R. A., C. L. SWANSON, D. WEISLEDER, AND I. M. CULL. Gutta-Producing Grasses. *Phytochemistry* 18 (1979):1069-1071.

BUCHANAN, R. A. AND F. H. OTEY. Multi-Use Oil- and Hydrocarbon-Producing Crops in Adaptive Systems of Food, Material, and Energy Production. *Biosources Dig.* (1979):176-202.

Reports:

BAGBY, M. O. Phytochemicals: Renewable Energy Resources. AES Collaborators Conference, NRRC, Peoria, Illinois, October 22-23, 1979.

BUCHANAN, R. A. Botanochemicals. Invited presentation 19th Annual Phytochemical Society of North America, Northern Illinois University, DeKalb, IL, August 13-15, 1979.

BUCHANAN, R. A. Multi-Use Crops and Botano-Chemical Production, Abstract of Papers, ACS/CSJ Chemical Congress: Cellulose, Paper, and Textile Division, Natural Products Symposium on Annual Plants, Tropical Woods and Underutilized Species, Honolulu, Hawaii, April 1979. Abstract 3.

BUCHANAN, R. A. Multi-Use Crops and Botanochemical Production. Invited presentation. The University of Tucson, Tucson, AR, April 16, 1979.

BUCHANAN, R. A. Multi-Use Botanochemical Crops and Botanochemical Production, 11th Central Regional Meeting, Columbus, OH, ACS, May 7-9, 1979.

BUCHANAN, R. A. Energy Plants. Invited presentation, 20th Annual Corn Dry Milling Conference, Peoria, IL, May 31, 1979.

Numerous interviews for trade journals, television, radio, and newspaper releases.

8. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. J. Bothast)

See Fermentation Laboratory, F. 1.

9. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

- a. Specific Objective: Develop an efficient process that converts aflatoxin-contaminated corn to alcohol and renders the spent grain safe for animal feed.

Progress: Zymomonas mobilis demonstrated greater fermentative activity than Saccharomyces uvarum during the first day in the fermentation of two lots of aflatoxin-contaminated corn and two corresponding lots of ammonia-detoxified corn. Final ethanol yields and conversion efficiencies were generally highest in Zymomonas fermentations of ammonia detoxified corn. Aflatoxin levels in post fermentation solids from ammonia-detoxified corn all ranged below the FDA feedstuff guideline of < 20 ppb, whereas, aflatoxin increased over initial levels in the post fermentation solids of aflatoxin contaminated corn.

When ammonia was applied at a rate of 0.5-2.0% d.b., as ammonium hydroxide to the fermentation mash of aflatoxin contaminated corn, the level of aflatoxin in the spent grains was markedly reduced. Studies are being concluded to optimize the level of ammonia addition as well as the reaction time and temperature to produce ethanol and render the spent grain safe for animal feed.

- b. Specific Objective: Select microorganisms that ferment sugars derived from chemical and biological processes to alcohols and/or other feedstocks.

Progress: A systematic search of yeasts, bacteria, and molds from the NRRC culture collection has begun for microorganisms that grow on xylose and for microorganisms that ferment xylose to ethanol or other useful chemicals. Pichia burtonii, Kluyveromyces fragilis,

and Pichia scolyti are yeasts which produced small amounts of ethanol on xylose.

- c. Specific Objective: Determine the effect of substrate and ethanol concentration and incubation time and temperature on yield and rate of alcohol production in comparative fermentation with traditional Saccharomyces cerevisiae strains, Zymomonas strains, and strains of Saccharomyces cerevisiae (sake yeast) which are extremely tolerant to ethanol.

Progress: S. cerevisiae utilizes all available glucose nearly 8 hours prior to a comparable culture of S. uvarum; however, both yeasts convert 100% of the glucose in the medium to alcohol within 48 hours. Zymomonas mobilis grew faster than the yeast cultures but took twice as long to completely convert the glucose to alcohol. Both the yeast and bacteria converted lower concentrations of sugar more efficiently than sugar concentration above 5.0%.

The specific growth rate (μ) of Z. mobilis decreased rapidly as ethanol concentration increased in batch fermentations of 10% (w/v) glucose. At 4% (w/v) ethanol, μ was only 1/10 the value of μ with no ethanol present.

- d. Specific Objective: Confirmation will be sought for an immobilized cell process for conversion of plant polymer sugar to alcohol.

Progress: Sodium alginate used to immobilize yeast cells in a continuous alcohol production experiment did not appreciably affect the viability of the two yeast species during the first week of cell immobilization. A gradual decrease in cell viability occurs between the second and fourth week after the cells have been immobilized in the alginate. About 50% of the yeast cells survive for two weeks immobilized in the alginate suspended in 10% glucose buffer solution. Yeast cells survive about 5 days in an optimal growth medium containing 10% glucose. Sixteen hour cultures of yeast cells were immobilized in Na-Ca-alginate gels and placed in a column. Continuous fermentations were conducted over periods up to 54 days with 10% glucose feed. One hundred percent conversion, i.e., 5% ethanol, was observed during the first 8 days. After 25 days, ethanol yields dropped to 2% and by 54 days, little glucose was converted to ethanol.

Reports:

BOTHAST, R. J. Innovative Fermentation Technology for Alcohol Production. Presented at the Conference of General Collaborators, Peoria, Illinois, October 22-23, 1979.

BOTHAST, R. J. Fermentation Aspects of Alcohol Production. Presented at the Annual Meeting of the Illinois Chapters, Soil Conservation Society of America on "Energy for the Eighties," Champaign, Illinois, November 1-2, 1979.

C. TECHNOLOGIES FOR FIBER USES

1. Storage and Processing of Kenaf for Pulp and Papermaking (M. O. Bagby)

- a. Specific Objective: Prepare mechanical, thermomechanical, and chemimechanical kenaf pulps.

Progress: An industrial cooperator (by hire) prepared several kenaf thermomechanical pulps at various temperatures and refiner energy inputs. Both variables were difficult to control due to mechanical feed problems. Consequently, 10-lb samples were collected for evaluation. Mechanical and chemimechanical (hydrogen peroxide) pulps were generated in a different refiner which allowed better control of refiner energy input. Several 10-lb samples were collected and about 400 lb of both mechanical and chemimechanical pulps were produced.

- b. Specific Objective: Evaluate kenaf thermomechanical, mechanical, and chemimechanical pulps and determine their suitability as newsprint furnishes.

Progress: Thermomechanical pulps were bleached and further refined by a secondary mechanical treatment. Paper formed from the pulp had properties similar to those of commercial newsprint. The mechanical pulp after bleaching to 68% brightness and secondary refining had strength properties equal to commercial newsprint. The chemimechanical pulp had an 85% brightness compared to 64% for commercial newsprint. This high brightness again reflects the facility with which kenaf bleaches. Except for a slightly lower tensile strength, the paper formed had strength properties similar to those of commercial newsprint.

- c. Specific Objective: Evaluate effect of sewage sludge applications to stripmined land on the growth of kenaf.

Progress: By Memorandum of Understanding, kenaf was grown in three replications of four treatments: as is, commercial fertilizer, and two levels of sewage sludge (25 ton and 50 ton per acre). Samples were harvested from randomized subplots 1 month after a killing frost to yield 3,100, 3,300, 2,800, and 2,100 pounds/acre, respectively. Plant densities on sludge

treated plots were significantly lower at the 95% confidence level.

Publications:

ADAMSON, W. C., F. L. LONG, AND M. O. BAGBY. Effect of Nitrogen Fertilization on Yield, Composition, and Quality of Kenaf. Agron. J. 71 (1979):11-14.

BAGBY, M. O., R. L. CUNNINGHAM, G. F. TOUZINSKY, G. E. HAMERSTRAND, E. L. CURTIS, AND B. T. HOFREITER. TAPPI Press Report, Nonwood Plant Fiber Pulping. Progress Report No. 10, September 1979, Atlanta, GA, p. 111.

CUNNINGHAM, R. L., G. F. TOUZINSKY, AND M. O. BAGBY. Brightening of Kenaf Thermomechanical Pulp. Tappi 62 (1979):69-70.

MOORE, C. A. Kenaf a Potential Pulp Crop. Staff Report National Economics Division (August 1979): 32 pp.

TOUZINSKY, G. F. AND S. H. GORDON. Degree of Substitution of Cellulose Derivatives Containing 11 Different Substituent Groups. Carbohydr. Res. 69 (1979):327-329.

TOUZINSKY, G. F., R. L. CUNNINGHAM, AND M. O. BAGBY. Papermaking Properties of Kenaf Thermomechanical Pulp. Tappi in press.

TOUZINSKY, G. F., R. L. CUNNINGHAM, AND M. O. BAGBY. Laboratory Papermachine Runs with Kenaf Thermomechanical Pulp. Tappi in press.

Reports:

BAGBY, M. O. Fiber from Annual Plant Sources. Invited presentation, 19th Annual Phytochemical Society of North America, Northern Illinois University, DeKalb, IL, August 13-15, 1979.

TOUZINSKY, G. F. AND M. O. BAGBY. A Search for New Fiber Crops: A Review of the Evaluation Procedure. Abstract of Papers, ACS/CSJ Chemical Congress; Cellulose, Paper, and Textile Division, Natural Products Symposium on Annual Plants, Tropical Woods and Underutilized Species, Honolulu, Hawaii, April 1979. Abstract 138.

TOUZINSKY, G. F. Guayule as a Source of Papermaking Fibers. Abstract of Papers, ACS/CSJ Chemical Congress: Cellulose, Paper and Textile Division Natural Products Symposium on Annual Plants, Tropical Woods and Underutilized Species, Honolulu, Hawaii, April 1979. Abstract 187.

Numerous interviews with technical journals, trade journals, and news media.

D. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. Principles Underlying Design of Food Blends for the Export Market (R. A. Anderson)

- a. Specific Objective: Continue development and evaluation of new cereal food products with improved nutritional quality.

Progress: The compatibility of added tricalcium phosphate (TCP) with various PL-480 commodities was tested. TCP was proposed as a potential insect retardant and tests were carried out to determine the extent of settling and dusting after addition. TCP was added at 1 and 2% to bulgur, soy-fortified bulgur, soy-fortified rolled oats, soy-fortified sorghum grits, soy-fortified bread flour, and soy-fortified corn meal. Dusting was a problem with all commodities tested. Settling was also a problem with all commodities except soy-fortified bread flour and soy-fortified corn meal. Both dusting and settling were minimized to insignificant levels by the addition of 1 part soybean oil to 2 parts TCP or 1.5 parts soybean oil to 2.7 parts CSM-type mineral premix. These PL-480 commodities with added TCP plus soybean oil are currently being tested for efficacy in retarding insects.

Studies underway on the use of microwave energy to rapidly destroy salmonella in corn-soy-milk (CSM) indicate the feasibility of such an approach to be valid. Preliminary tests are establishing processing parameters for conducting the treatment on CSM which has been bagged and ready for shipment. Concern over the possible long-term retention of heat in the treated bags and possible nutritional deterioration has prompted research into the effects of different ways of palletizing and storage on these factors. The treated products are being evaluated for salmonella destruction relative to nutritional change.

- b. Specific Objective: Investigate the extrusion process as a means of altering structural properties of cereals and cereal products to produce new food products.

Progress: Basic rheological studies on corn and wheat flour doughs using the mechanical spectrometer are showing that the elastic modulus and the viscous modulus can be used as parameters for evaluating effects of shear rates and mechanical stresses on the doughs. This instrument utilizes eccentric rotating discs

which are rotated in the range of 0.1 to 225 radians per second. Doughs were prepared from wheat flours extracted from durum, hard red winter, hard red spring, soft red and white winter wheats by mixing flour and water in proportions to give a farinograph value of 500 Brabender units. Moisture contents varied from 36 to 48%. In general, both moduli increased over the time scale. Addition of soy flour (6% and 12%) to hard red winter wheat flour results in increases in the elastic modulus ranging from about 75 to 250% over control using the same time scale, and increases in the viscous modulus of 100 to 200%.

Publications:

BOOKWALTER, G. N., R. J. BOTHAST, W. F. KWOLEK, AND M. R. GUMBMAN. Nutritional Stability of Corn-Soy-Milk Blends After Dry Heating to Destroy Salmonella. J. Food Sci. Accepted for publication, January 12, 1980.

BOOKWALTER, G. N., K. WARNER, R. A. ANDERSON, AND E. B. BAGLEY. Peanut Fortified Food Blends. J. Food Sci. 44 (1979):820-825.

Reports:

BOOKWALTER, G. N. "Foods for Small Children." Spring Training Program, Meals for Millions Foundation, Santa Monica, California, May 1979.

BOOKWALTER, G. N. Symposium Chairman, "Extrusion Cooking of Cereals and Cereal Components." Annual Meeting, American Association of Cereal Chemists, Washington, D.C., October 1979.

CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

Research Leaders: J. E. Hodge and J. S. Wall

A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Enzyme Modification of Feed and Cellulosic Materials for Improved Beef Animal Feed (H. L. Griffin)

- a. Specific Objective: Investigate reverse osmosis as a technique to selectively retain the enzyme co-factors of Trichoderma reesei cellulase in its digests with cellulose as the sugar products are being removed.

Progress: Investigations involving non-cellulosic reverse osmosis membranes indicate that the swelling factor (Cl) and other co-factors that control the efficiency (rate and extent) of the cellulolysis as well as the average degree of polymerization (DP) of the product are only partially retained by Amicon UM-05 membranes that retain only those organic molecules possessing a nominal DP of 500 or are positively charged in the digest environment. As a result the efficiency of the cellulase complex remaining in the digest is typically reduced 50% by the simultaneous removal of co-factors and sugar products through the membrane. Amicon membranes that pass protein molecules with a molecular weight of 10,000 or above will allow some or all of the enzymes in the cellulase complex to contaminate the product as well.

- b. Specific Objective: Investigate the effect of plant variety and its geographical source on the neutral sugar content of the cellulose and hemicellulose components of the stalk and stalk fractions (rind and fibrovascular bundles) of corn, sugarcane, sweet sorghum, and sunflowers.

Progress: There are only minor differences in the neutral sugar composition of the cellulose and semicellulose components isolated from the stalk, rind, and fibrovascular bundles of corn, sugarcane, sweet sorghum and sunflowers grown at various locations. However, varietal differences especially in xylose content (concentrated mainly in the hemicellulose A component) vary widely. For example, the sugarcane stalks nearly 25% xylose and corn 20% whereas sweet sorghum is 17% xylose and sunflower is only 2%. The data is useful for evaluating these residues as sugar sources.

Publications:

JONES, R. W., L. H. KRULL, C. W. BLESSIN, AND G. E. INGLETT. Neutral Sugars of Hemicellulose Fractions of Pith from Stalks of Selected Plants. Cereal Chem. 56 (1979):441-442.

KRULL, L. H. AND G. E. INGLETT. Analysis of Neutral Carbohydrate in Agricultural Residues by Gas-Liquid Chromatography. J. Agric. Food Chem. (submitted for publication).

2. Molecular Structure of Cereal-Derived Sugars, Sugar-Derived Compounds, and Their Complexes (J. E. Hodge)

- a. Specific Objective: With the similar structures of naturally occurring sweet compounds as models, synthesize compounds to obtain more information on structure-taste relationships.

Progress: Continued syntheses of compounds with chemical structures closely related to naturally occurring phyllodulcin have produced 2-(3-hydroxy-4-methoxyphenyl)-1,3-dioxole, four flavans and their parent flavanones that provide new information on stereochemical requirements for compounds of this group to elicit an intensely sweet taste. Intensely sweet taste is given by those compounds with highly specific orientations of functional groups that can adopt a bent conformation at the receptor site, although their conformations in solution are semiplanar. Conformational rigidity in a closely related semiplanar molecule diminished sweetness twentyfold and greatly increased bitterness.

The sugar degradation products, maltol and isomaltol (flavor compounds and sweetness enhancers) formed sodium-free 3:1 crystalline complexes with sodium aluminate in aqueous solutions.

The various and multiple reactions of methanesulfonate esters of glucosides with base that produce mono- and di-anhydro derivatives were rationalized in a comprehensive study of the transformations of six methyl di-O-methanesulfonyl- α -D-glucopyranosides. These reactions convert D-glucose into derivatives of known and fixed conformations that are useful in defining the molecular complexing reactions of sugars.

Publications:

DICK, W. E., JR. Efficiency of Cadmium Carbonate as an Aryl Glycosidation Catalyst: Effects of Lot Variations on Product Compositions. Carbohydr. Res. 70 (1979):313-318.

GOODWIN, J. C., J. E. HODGE, AND D. WEISLEDER. Preparation of Bicyclic Hexitol Anhydrides by Using Acidic Cation-Exchange Resin in a Binary Solvent. Carbohydr. Res. In press.

MILLS, F. D. Vacuum Thermolysis of 1-deoxy-1-Sarcosino-D-Fructose. J. Agric. Food Chem. 27 (1979):1136-1138.

RENDLEMAN, J. A., JR. AND J. E. HODGE. Complexes of Carbohydrates with Aluminate Ion Aldose-Ketose Conversion on Anion-Exchange Resin (Aluminate and Hydroxide Forms). Carbohydr. Res. 75 (1979):83-99.

SINCLAIR, H. B. Action of Bases On the di-O-Methanesulfonyl Esters of Methyl α -D-Glucopyranoside: Displacement Order and End Products. J. Org. Chem. 44 (1979):3361-3368.

SINCLAIR, H. B. Conformation of 1,3:4,6-di-O-chloroethylidene Galactitol and its Conversion into 1(R*), 4(S*)-di-O-Ethylidenegalactitol. Carbohydr. Res. 70 (1979):243.

VAN CLEVE, J. W. Reinvestigation of the Preparation of Cholesteryl 2,3,4,6-tetra-O-Benzyl- α -D-Glucopyranoside. Carbohydr. Res. 70 (1979):161.

Reports:

HODGE, J. E. Carbonyl-Amine Reactions and Their Effects on Proteins. Presented at ACS/CSJ Chemical Congress, Honolulu, Hawaii, April 1-6, 1979.

HODGE, J. E. AND F. D. MILLS. Polymeric Products Formed by Hydrolysis of Amino-Hexose-Reductones. Presented at the International Symposium, Maillard Reactions in Food, Uddevalla, Sweden, September 2-6, 1979.

MILLS, F. D. AND J. E. HODGE. 1,6a,11a,11b-Tetrahydro-5,7,8-Trihydroxy-3,6a,10,11b-Tetramethyldiindeno (7,1-bd:2,1e)pyra-2,11-dione, A Condensation Product Formed by Hydrolysis of Amino-Hexose-Reductones. Presented at 178th ACS National Meeting, Washington, D.C., September 10-14, 1979.

SINCLAIR, H. B. Action of Base on the Di-O-Methanesulfonyl Esters of Methyl α -D-Glucopyranoside: Displacement Order and End Products. Presented at 178th ACS National Meeting, Washington, D.C., September 10-14, 1979.

3. Functional Properties of Proteins in Wheat and Related Grains, Their Flours, and Protein Isolates (J. S. Wall)

- a. Specific Objective: To confirm and extend knowledge on sites of synthesis and deposition of wheat endosperm proteins.

Progress: Immature wheat kernels were examined by transmission electron microscopy at various times of maturity from 7 to 35 days after flowering. Protein bodies were found as early as 14 days

(possibly even 7 days) and disappeared by approximately 25-30 days after flowering. Additional work was conducted to determine the composition of these protein bodies and possibly what transformations they undergo. Immature wheat grains which had been homogenized in isotonic buffer and subjected to sucrose density gradient ultracentrifugation were further analyzed for protein content and composition. Automated ninhydrin analysis with alkaline hydrolysis was used to monitor protein distributions among the density gradient fractions, and protein-rich fractions were analyzed by polyacrylamide gel electrophoresis in aluminum lactate buffer (to detect gliadins, albumins, and globulins) and by SDS-electrophoresis (to analyze glutenin and also total protein contents). Initial results have revealed some albumins of globulins in less-dense, upper density gradient fractions, as well as some crosslinked protein which may be high-molecular-weight gliadin distributed evenly across the gradient. The majority of characteristic gliadin and glutenin electrophoretic bands were not detected, suggesting that these proteins were pelleted under the homogenization/centrifugation conditions used.

- b. Specific Objective: Determine factors responsible for improvement in baking quality in grain stored several months.

Progress: Proteins extracted from freshly harvested wheat and wheat stored for 7 months were compared. Changes in the quantity of the different proteins extracted were apparent. By using an alkylating agent in the extracting solvent, disulfide bonding during extraction was prevented. Results indicate that some of the higher molecular weight glutenins are not yet disulfide bonded when the wheat is fresh. Some indication was also found indicating disulfide bonding took place during normal extraction procedures.

Publications:

HUEBNER, F. R. AND J. S. WALL. Polysaccharide Interactions with Wheat Proteins and Flour Doughs. *Cereal Chem.* 56(2) (1979):68-73.

HUEBNER, F. R. AND J. S. WALL. Wheat Glutenin: Effect of Dissociating Agents on Molecular Weight and Composition as Determined by Gel Filtration Chromatography. *J. Agric. Food Chem.*, in press.

WALL, J. S. Properties of Proteins Contributing to Functionality of Cereal Foods. *Cereal Foods World* 24 (1979):288-292, 313.

WU, Y. V. AND A. C. STRINGFELLOW. Protein Concentrate from Air Classification of High-Protein Soft Wheat Flours. *J. Food Sci.* 44 (1979):453-455.

WU, Y. V., K. R. SEXSON, AND J. E. SANDERSON. Barley Protein Concentrate from High-Protein, High-Lysine Varieties. J. Food Sci. 44 (1979):1580-1583.

Reports:

HUEBNER, F. R. Chemical Changes Within Wheat Proteins During Storage of the Whole Wheat. Presented at 64th Annual AACC Meeting, Washington, D.C., October 28-November 1, 1979.

WU, Y. V., K. R. SEXSON, AND J. E. SANDERSON. Barley Protein Concentrate from High-Protein, High-Lysine Varieties. Presented at 39th Annual Meeting of the Institute of Food Technologists, St. Louis, Missouri, June 10-13, 1979.

WU, Y. V. Protein Concentrates from Oat Flours by Air Classification and by Density Separation in Nonaqueous Solvents. Presented in the symposium "Physical Separations in Food Processing," 87th National American Institute of Chemical Engineers Meeting, Boston, Massachusetts, August 19-22, 1979.

4. Preparation of Protein Products from Corn and Related Cereals (J. S. Wall)

- a. Specific Objective: Study the effect of germination on the amino acid composition and protein fractions of normal and high-lysine sorghums.

Progress: Sorghum is deficient in lysine for humans. Normal and high-lysine sorghums were germinated for 1 to 10 days. Lysine content of germinated normal sorghum increased from 2.2 to 3.1 g per 16 g nitrogen after 6 days. For sprouted high-lysine sorghum, lysine content increased from 3.0 to 7.8 g per 16 g nitrogen after 7 days. A large increase in albumin fraction (rich in lysine) and a large decrease in kafirin and crosslinked kafirin fractions (both low in lysine) accompanied sprouting. The percent protein in germinated sorghum is greater than in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of protein per kernel is not increased.

- b. Specific Objective: To develop physical and chemical means of concentrating and separating protein and other valuable nutrients from grain alcohol fermentation stillage.

Progress: The stillage from grain alcohol fermentation was separated into various fractions by screening and centrifugation. Preliminary work indicates that the soluble fraction consists of relatively small molecules (molecular weight less than 10,000), and the solid fractions were considerably less soluble in the typical solvents used for fractionation of corn proteins.

- c. Specific Objective: Determine chemical and physical properties of zeins.

Progress: The water-insoluble zein was extracted from corn meal with 70% ethanol-0.5% sodium acetate and then fractionated with 95% ethanol into soluble α -zein and insoluble β -zein components. Electrophoresis in sodium dodecyl sulfate established that β -zein has very high molecular weight (MW) while α -zein consists of lower MW proteins; lowest MW being 24,000. When their disulfide bonds are cleaved by reduction, both α and β -zeins yield polypeptide subunits with MW's 22,000 and 24,000. These subunits are separated into over 15 different polypeptides by isoelectric focusing. Zein from high-lysine corn has less of the 24,000 subunits than that from normal corn. These results establish that the solubility of zein in alcohol dependent on the degree of intermolecular disulfide crosslinks and explains many of native zeins properties.

Publications:

NIELSEN, H. C., J. S. WALL, AND G. E. INGLETT. Flour Containing Protein and Fiber Made from Wet-Mill Corn Germ, with Potential Food Use. Cereal Chem. 56(3) (1979):144-146.

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PAULIS, J. W. Zein Proteins from Corn Endosperm. Presented at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, November 1, 1979.

PAULIS, J. W. Zein Proteins for Commercial Applications. Presented at Grain Processing Corporation, Muscatine, Iowa, November 15, 1979.

WALL, J. S. Maximizing Use of Normal and High Lysine Sorghums in Foods. Eleventh Biennial Grain Sorghum Research and Utilization Conference, Wichita, Kansas, February 28-March 2, 1979.

5. Interactions of Food Carbohydrates (J. E. Hodge)

- a. Specific Objective: Determine the mineral binding properties of the water-soluble and water-insoluble fractions of hard red spring wheat bran and compare results with data on the soft white wheat bran already analyzed.

Progress: Comparisons of the binding capacities of hard red spring wheat bran and soft white wheat bran for calcium and zinc ions showed only small differences between the two under varying conditions of pH and heat treatment. Zinc is bound six to eight times more strongly than calcium which is bound more strongly than strontium. One-half gram of soft white wheat bran will remove 92% of the zinc from 100 ml of 0.002M zinc chloride solution at pH 7.2, 37°C; 63% from the same solution at pH 6.4 and 62% at pH 4.5. The aqueous extract of bran also has a great binding power for zinc which is pH dependent. The major binding constituent in aqueous bran extract was found to be phytate ion. When calcium ion was added to an aqueous extract, a highly insoluble calcium complex was isolated which contained 64% calcium phytate, 14% protein (%N X 5.95), and 14% water of hydration, and 8% of other constituent(s) not determined. The pectic substances bound in the bran, already chelated with mineral ion(s), react only weakly with calcium and zinc; however, pure polygalacturonic acid binds calcium, zinc, and strontium moderately well, and this binding is only slightly influenced by pH. Human saliva does not bind zinc significantly, but gastric mucin binds moderately well and bovine serum albumin binds slightly. Both phosphate and phytate ions bind zinc very strongly at pH 6-7. Cellulose, starch, and glucose do not bind zinc significantly in the pH range 4.4-6.9. This study contributes to knowledge of the physiological effects of dietary fiber in humans.

6. Corn Starches--Physical Characteristics and Biological Digestibilities
(G. E. Inglett)

- a. Specific Objective: To determine the gelatinization ranges and alpha-amylase digestibility of fractionated starch granules of amylomaize V, amylomaize VII, waxy starch, and ordinary starch.

Progress: Enzyme hydrolysis rate studies of unfractionated granules and sized fractions of Amaizo 100 starch were completed. Enzyme digestibility of starches by pancreatic α -amylase were conducted on raw granules and partially gelatinized starches Amaizo 100, waxy maize, and Amylomaizes V and VII. The Amylomaize V and VII show better resistance to enzyme corrosion than other types surveyed. Partially gelatinized samples react more readily with enzymes but still not as fast as raw dent or the waxy maize starch. The small granules of dent and waxy react faster than do the large ones. This is not true for amylomaizes. With the amylomaizes there is very little enzyme attack prior to gelatinization. SEM techniques have been surveyed and used in viewing the four starches. Enzyme attack was followed during digestions with light and electron microscopy. Amylomaize VII intermediate size granules are much more resistant to the enzyme than either the large or the small granules. This pattern was observed both on the raw and partially gelatinized samples. No correlation was found between amylose content and enzyme digestibility.

Gelatinization ranges, Birefringent End Point Temperatures (BEPT), were measured on the four parent starches and on selected sized fractions from them. These studies showed that the refractivity of the intermediate sized Amylomaize VII particles was greatest. A correlation may be seen between BEPT and enzyme activity as to refractivity of these granules.

Study of in situ starch in mutants was continued on embedded endosperm material. For acquiring basis material for the characterization work, starch extraction from line B-37 (NU) corn and several mutants was carried out using a mercuric chloride steep.

Publication:

KNUTSON, C. A., J. E. CLUSKEY, AND G. E. INGLETT. Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules. Staerke/Starch 3 (1980).

Report:

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7. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn
(G. E. Inglett)

See Biomaterials Conversion Laboratory, A.3.

8. Interactions of Dietary Fibers from Cereal Products with Mutagens in Digested Food (J. Lehrfeld)

- a. Specific Objective: Determine whether any of the different types of dietary fiber in controlled human diets are instrumental in removing potential carcinogens from the digestive tract.

Progress: The necessary cultures for performing the Ames tests for mutagenic activity were obtained from the NRRC Fermentation Laboratory, recultured, plated, and checked for viability and sensitivity to ultraviolet irradiation. A gas-liquid chromatographic method for the simultaneous analysis of the neutral and acidic carbohydrates in any polysaccharide fraction of dietary fiber found to interact with a mutagen was developed.

9. Methods of Analysis to Facilitate Genetic Improvements of Cereal Grain Protein (J. S. Wall)

- a. Specific Objective: Determine genetic relationships of cereals and their proteins through amino acid sequence analyses.

Progress: Prolamin fractions from barley, oats, normal and high lysine sorghums, Teosinte and Tripsacum were subjected to additional N-terminal amino acid sequence analyses. High-performance liquid chromatographic analyses methods were adapted and computerized to permit greatly increased speed and accuracy in characterizing sequencer fractions. Comparison of N-terminal sequences of high molecular weight gliadin and ethanol-soluble reduced glutenin by sequence analyses has proven their identity, leading to a hypothesis of the origin and interactions of each type of wheat protein. Attempts to sequence high-molecular-weight ethanol-insoluble glutenin subunits, which have blocked N-terminus, after treatment with pyrrolidonecarboxyl peptidase have failed, suggesting that the N-terminal amino acid is not pyroglutamic acid. A low-molecular-weight, high-methionine fraction of alcohol-soluble corn glutelin was also found to resist sequence analysis, and apparently has a blocked N-terminus. Fractions of purified alcohol-soluble corn glutelins having higher molecular weights were obtained from A. Esen (Virginia Polytechnic Institute) and subjected to sequence analyses; highly unusual sequences were obtained. Following an initial N-terminal sequence of 11 residues, a 6-residue sequence was found which repeated six or more times.

- b. Specific Objective: Analyze proteins to establish a basis for improving grain quality in experimental wheat and triticales breeding programs.

Progress; Nine pairs of Triticale lines which had either opaque or vitreous endosperms were extensively characterized by electrophoresis; gliadins, glutenins, and the total protein contents were examined. Heterogeneity of some samples was detected, but consistent differences in protein distribution between the two endosperm types did seem to occur.

Two high molecular weight glutenin subunit bands in durum wheat are coded by chromosome 1B, and may be associated with pasta or bread-making quality. Dr. L. Joppa, SEA-AR, Fargo, ND, is attempting to substitute, through breeding, 1B chromosomes from other varieties into Langdon durum as a means of improving quality. Many such breeding attempts have been analyzed by various electrophoresis techniques in order to determine protein bands regulated by 1B chromosomes from different varieties; once optimum 1B chromosome sources have been identified, electrophoresis can then be used as a selection tool for transferring good quality characteristics into new varieties. In addition, electrophoresis has been used to study other durum wheat aneuploids, addition lines, and substitution lines to complement cytological techniques and to establish banding patterns for each chromosome; such studies are still underway.

In conjunction with these studies, a new method was developed for first isolating gliadins from single kernels of cereal grains and characterizing them by electrophoresis in aluminum lactate buffer, and then extracting glutenin from the residue of the same kernels and characterizing it by SDS-electrophoresis. Germ ends of kernels can be retained and used for cytological chromosome analysis or can be grown into mature plants.

- c. Specific Objective: To locate the genes responsible for coding zein and glutelin proteins on chromosomes of corn.

Progress: Because of limited availability of grain and variations among kernels in genetic studies, a simple and inexpensive mill was designed to pulverize a single or half kernel of cereal grain in a short time. Previous methods of grinding single kernels of wheat, corn, or sorghum were tedious or not quantitative. Since breeders and geneticists need to use only the germ portion of the seed for growth of the new plant on agar media, the endosperm tissue can be ground and analyzed for prolamine proteins. Zeins in extracts of the ground endosperm of a single kernel of corn were analyzed by isoelectric focusing on polyacrylamide gels to demonstrate the effectiveness of single kernel analyses in establishing genetic differences.

Publications:

BIETZ, J. A. AND J. S. WALL. Identity of High-Molecular-Weight Gliadin and Ethanol-Soluble Glutenin Subunits of Wheat: Relation to Gluten Structure. Cereal Chemistry, submitted for publication.

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BIETZ, J. A. Recent Advances in the Isolation and Characterization of Cereal Proteins. Cereal Foods World 24 (1979):199.

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WALL, J. S. Role of Wheat Proteins in Determining Baking Quality. In "Recent Advances in the Biochemistry of Cereals," edited by D. L. Laidman and R. G. Wyn-Jones, Academic Press, London, 1979, pp. 275-311.

Reports:

BIETZ, J. A. Relation Between High-Molecular-Weight Gliadin and Ethanol-Soluble Reduced Glutenin of Wheat Demonstrated by Sequence Analysis. Presented at 64th Annual AACC Meeting, Washington, D.C., October 28-November 1, 1979. Abstract published in Cereal Foods World 24 (1979):453.

BIETZ, J. A. Cereal Protein Research. Seminar presented at Virginia Polytechnic Inst., Dept. of Biology, Blacksburg, Virginia, November 2, 1979.

10. Epoxides from Lipid Hydroperoxides and Their Interactions in Cereal and Oilseed Foods (H. W. Gardner)

- a. Specific Objective: Study the pathways of formation and degradation of fatty acid epoxides from fatty acid hydroperoxides and assess the mutagenicity/toxicity of fatty acid hydroperoxides and epoxides by the Ames test.

Progress: Homolytic decomposition of hydroperoxides of polyunsaturated fatty acids often results in fatty epoxides, which are potentially toxic compounds. The decomposition of a specific fatty acid hydro-

peroxide, 13-L(S)-hydroperoxy-cis-9, trans-11-octadecadienoic acid, yielded a number of epoxides among which was a novel, optically active compound, 11-oxotrans-12,13-epoxy-trans-9-octadecenoic acid. The discovery of this epoxide filled a gap in the theory of epoxide formation. Previously, the presence of this epoxide was unsuspected, because the epoxide was mixed with a number of other compounds; however, column chromatography in combination with high pressure liquid chromatography was successful in achieving isolation. Also isolated was 9-hydroxytrans-12,13-epoxytrans-10-octadecenoic acid which was suspected to be present, but not isolated previously due to its tendency to form other products. This latter epoxide was a diastereomeric pair. The pair was separated by either thin-layer chromatography or high pressure liquid chromatography, and the stereoisomerism of the isolates was confirmed by Dr. C. P. A. van Os at the University of Utrecht. Additionally, relatively large samples of five different fatty epoxides were prepared for assessing mutagenicity by the Ames Test. A sample of 99+% pure methyl 13-L(S)-hydroperoxy-cis-9, trans-11-octadecadienoate also was prepared for testing by the method of Ames. The Ames Test will be done at SEA-AR's Western Regional Research Center, Albany, CA.

Using a cysteine-FeCl₃ catalyst to degrade linoleic acid hydroperoxides, it was found that under alkaline conditions the hydroperoxide was reduced to a hydroxyl group and superoxide anion was generated. Under alkaline conditions no epoxides were produced.

Publications:

GARDNER, H. W. AND R. KLEIMAN. Lack of Regioselectivity in Formation of Oxohydroxyoctadecenoic Acid from the 9-or 13-Hydroperoxide of Linoleic Acid. *Lipids* 14 (1979):848.

GARDNER, H. W. Lipid Enzymes: Lipases, Lipoxygenases, and "Hydroperoxidases." In "Autoxidation in Food and Biological Systems," edited by M. Simic and M. Karel, Plenum Press, N.Y. (in press).

Reports:

GARDNER, H. W. Lipid Enzymes: Lipoxygenases, Lipases. Pre-convention symposium on Food Lipids, IFT Meeting, St. Louis, Missouri, June 8-9, 1979.

GARDNER, H. W. Lipid Hydroperoxide Degradation. Workshop on Autoxidation Processes in Food and Related Biological Systems. U.S. Army NARADCOM, Natick, Massachusetts, October 29-31, 1979.

11. Molecular Structure of Maillard-Type Browning Reaction Products (J. E. Hodge)

- a. Specific Objective: Conduct sugar-amine browning reactions to produce and isolate several fractions of the many soluble reaction products for compositional analysis, so that detailed knowledge will be obtained on the nonvolatile products of Maillard-type browning reactions in processed foods.

Progress: Eleven different fractions of nondialyzable browning polymers were prepared from sugar-amino acid reactions. Isomaltol (2-acetyl-3-hydroxyfuran, $C_6H_6O_3$), an acidic colorless sugar-amine browning reaction product, was found to decompose to brown polymers of low molecular weight during storage at ambient temperatures. The decomposition was produced more rapidly in weakly and strongly acidic aqueous media at low temperatures. Because progressive color changes of the isomaltol decompositions exactly resemble those of Maillard-type browning reactions, compositions and structures of the first-formed colored degradation products were determined. Various colored (yellow, greenish-yellow, orange, red, brown) new crystalline oxonium salts of five different strong acids were isolated and converted by washing with water to the same red-orange, crystalline dye, $C_{10}H_8O_4$, in 21% overall yield. The red-orange compound undergoes further browning decompositions in reactions with acids, amine salts, and amino acids to produce dark polymers of low molecular weight. These polymers were retained for future investigations. Knowledge of isomaltol decompositions, along with those of the isomeric 5-(hydroxymethyl)furfural being studied elsewhere, should reveal the two main routes to melanoidin formation in Maillard-type browning reactions of foods.

- b. Specific Objective: Isolate and determine the chemical structures of the most active antioxidants in fractions of sugar-amine reaction mixtures to protect unsaturated lipids from autooxidation.

Progress: A rapid method for analyzing the antioxidant activity of microgram amounts of browning reaction products was developed. The technique involves the hemoglobin-catalyzed oxidation of unsaturated fatty acid glycerides and following the inhibition of oxidation with a Pt/Ag/ O_2 electrode. This method correlates with the slower bulk oil analyses. Piperidino-hexose-reductone derived from a sugar-amine reaction was shown to be as active as butylated hydroxytoluene (BHT). Two non-nitrogenous reductones, $C_{12}H_{12}O_6$ and $C_{22}H_{20}O_6$, produced by hydrolysis of amino-hexose reductones, were only slightly less reactive on a weight basis. A browning reaction mixture obtained from D-glucose and DL-alanine was separated into neutral, cationic, and anionic fractions. The antioxidant activities of all three fractions were approximately equal to that of propyl gallate. Because these fractions were multicomponent, the antioxidant

activity of the specific active compounds present should be greater than that found for the mixture.

B. CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

1. Effect of Environmental Contaminants on Cereal Foods and Feeds (W. J. Garcia)

- a. Specific Objective: Complete research to determine the effects of stress on corn plants from ambient ozone generated by air pollution at chronic ozone levels, with special emphasis on changes in composition and nutritional quality of the edible grain.

Progress: Chronic doses of ozone in photochemical oxidant air pollution can cause foliar injury and decrease yield of important crop species; however, little is known about compositional changes in the edible portions of these crops as a result of plant exposure to ozone. For corn, threshold ambient ozone concentrations for foliar injury was between 0.02 and 0.07 ppm, but the threshold for decreased kernel yield was between 0.11 and 0.15 ppm. This study examined compositional data for the grain from a commercial field corn hybrid exposed to five levels of ozone ranging from 0.02 to 0.15 ppm, and two other single cross hybrids which were more sensitive to ozone but exposed at levels of 0.02, 0.06, and 0.15 ppm O₃. Compositional analysis of the grain included: protein, fat, ash, fiber, starch, amylose content of the starch, amino acid composition of the protein, and mineral analysis of macro and micro elements.

It was difficult to discern differences in composition for most analyses conducted, but the threshold for trace mineral elements was obtained at 0.15 ppm O₃ for the three corn varieties as evidenced by significant increases in metal contents for zinc, copper, iron, and especially cadmium. Varietal response to ozone was different in that the effects were greater for the two single cross hybrids.

- b. Specific Objective: Determine, from an extensive study with the Greater Peoria Sanitary District, the overall benefits and risks of anaerobically digested sludge as an amendment for diverse soil materials ranging in scope from disturbed residual materials such as coal mine gob and landfill to fully productive agricultural soils serving as growth media for a wide variety of edible crops.

Progress: Results of this completed work include data on the following aspects: (1) physical characteristics of soils as a result of their amendment with sludge, (2) yields for 15 different crops, (3) nutritional quality of selected crops, (4) metal uptake and accumulation in crop tissues, and (5) translocation of metals from soil medium to tissues.

Major general conclusions derived from the study were: (a) Sludge amendment of the soil materials ameliorated the soil pH in that pH values were moved nearer to neutral both from soils originally acid or alkaline. For example, growth of vegetation was virtually nonexistent in coal mine gob at a pH of 3.3; however, amendment of the gob resulted in a soil pH of 4.6 and permitted growth of all crops except eggplant. (b) Levels of metals in 14 different soil treatments varied widely with the highest levels of lead, cadmium, and zinc occurring in the landfill treatment, while the highest level of mercury was found in the sludge-only treatment. (c) Increased crop yields resulted with most sludge amended soils. The highest crop yields were obtained with both unamended and amended landfill and with black top soil. (d) Harvested crops with the highest metal content were derived from landfill and coal mine gob treatments, the lowest were associated with loam, clay, and top soils; the sludge-only treatment produced intermediate metal levels in these crops.

Lettuce accumulated high concentrations of metals and thus clearly reflected metal levels in the soil medium. By contrast, tomatoes had low levels of metals throughout all treatments and can be recommended as a desirable crop in disturbed or sludge amended soils. Metals in wheat were more concentrated in the vegetative parts of the plant than in the grain, with the exception of zinc which was more concentrated in the grain. This study showed that wheat grain can be expected to contain approximately 40% of the cadmium found in the wheat plant, thus levels of cadmium in grain could conceivably be elevated when plants are grown in soil materials high in cadmium. For this reason, corn may be a better grain crop to grow in disturbed or sludge amended soils because excessive quantities of metals are excluded from the kernels by the corn plant.

Publications:

BLESSIN, C. W. AND W. J. GARCIA. Heavy Metals in the Food Chain by Translocation to Crops Grown on Sludge-Treated Strip Mine Land. In Utilization of Municipal Sewage Effluent and Sludge on Forest and Disturbed Land. Edited by W. E. Sopper and S. N. Kerr, the Pennsylvania State University Press, pp. 471-482, 1979.

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GARCIA, W. J., C. W. BLESSIN, H. W. SANDFORD, AND G. E. INGLET. Translocation and Accumulation of Seven Heavy Metals in Tissues of Corn Plants Grown on Sludge-Treated Strip-Mined Soil. J. Agric. Food Chem. 27 (1979):1088-1094.

Report:

BLESSIN, C. W., W. J. GARCIA, J. F. CAVINS, AND G. E. INGLET, Northern Regional Research Center, Peoria, IL and A. S. HEAGLE, AR, SEA, USDA, North Carolina State University, Raleigh, NC. Effect of Atmospheric Ozone on Physical and Chemical Characteristics of Three Field Corn Hybrids. Presented at 64th annual American Association of Cereal Chemists' Meeting, October 28-November 1, 1979, Washington, DC.

C. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Loss of Mycotoxin in Corn During Storage or Ammonia Treatment (H. W. Gardner)

- a. Specific Objective: Isolate and characterize lipid hydroperoxides and products of their degradation. Assess these compounds for their toxicity/mutagenicity by the Ames Test.

Progress: The structures of the potentially mutagenic fatty acid epoxides obtained from fatty acid hydroperoxides were studied. It was discovered that the formation of these epoxides is regio- and stereospecific, i.e., 13-L-hydroperoxylinoleic acid degraded into optically active 12,13-epoxides. Other structural characteristics of the epoxides studied were erythro-threo isomerism and diastereoisomerism. A number of different complexes of iron were investigated for their ability to transform fatty acid hydroperoxides into fatty acid epoxides. One iron complex was observed to produce superoxide anion, but it was proved that superoxide had no role in the formation of fatty acid epoxides. One isomer of a hydroxyepoxyoctadecenoic acid was the precursor of an oxohydroxyoctadecenoic acid, evidently being formed via an "NIH Shift" reaction. Initial results of a cooperative project with WRRRC indicated that two fatty acid epoxide isomers tested are not mutagenic by the Ames test. An extensive review of the literature resulted in two review articles. The stereochemistry of product formation by corn linoleic acid hydroperoxide isomerase was established.

D. FOOD COMPOSITION AND IMPROVEMENT

1. Action of Human Digestive System upon Cereal Grain Fiber Sources and Related Foods (F. R. Dintzis)

- a. Specific Objective: Cooperate with the SEA-AR Human Nutrition Research Laboratory to correlate effects of dietary fiber type and composition with diet and physiological effects upon humans.

Progress: We continue to supply desired fiber sources to HNRL as required and have processed a large, second batch, of demineralized

bran. Remnants of brans have been retrieved from fecal matter and composition compared with that of starting material.

Publications:

DINTZIS, F. R., L. M. LEGG, W. L. DEATHERAGE, F. L. BAKER, G. E. INGLET, R. A. JACOB, S. J. RECK, J. M. MUNOZ, L. M. KLEVAY, H. H. SANDSTEAD, AND W. C. SHUEY. Human Gastrointestinal Action on Wheat, Corn, and Soy Hull Bran--Preliminary Findings. Cereal Chem. 56(3) (1979a):123.

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MILTON, K. AND F. R. DINTZIS. Nitrogen-to-Protein Conversion Factors for Tropical Plant Samples. Biotropica (in press).

MUNOZ, J. M., H. H. SANDSTEAD, R. A. JACOB, G. M. LOGAN, JR., S. J. RECK, L. M. KLEVAY, F. R. DINTZIS, G. E. INGLET, AND W. C. SHUEY. Effects of Some Cereal Brans and Textured Vegetable Protein on Plasma Lipids. Am. J. Clin. Nutr. 32 (1979):580.

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FERMENTATION LABORATORY

C. W. Hesseltine, Chief

Research Leaders: R. W. Detroy, M. D. Grove, T. G. Pridham,
O. L. Shotwell, and M. E. Slodki

A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Relationships of Nitrogen Fixation in Microorganisms and Plants to Reduce Energy Requirements of Crops (J. W. Newton)

- a. Specific Objective: To study any unique proteins elaborated during development of the Azolla-blue-green algal nitrogen-fixing symbioses.

Progress: Using polyacrylamide gel electrophoresis, the total protein constituents of the water fern Azolla and algae isolated from the symbiotic association were examined. In addition, algae grown under heterotrophic and autotrophic conditions and also on ammonia and molecular nitrogen were examined. These studies permitted detection of a relatively high molecular weight (ca 100,000) protein present in the fern during elaboration of the symbiosis. The nature of this constituent and its formation is under further investigation using radioisotopes.

- b. Specific Objective: Isolate and characterize algae from other Azolla species and study the physiology of these new strains.

Progress: Species of Azolla were collected from around the world by a cooperating scientist at the University of Hawaii.

- c. Specific Objective: The mechanism by which metronidazole (MNE) inhibits N_2 fixation and H_2 production (from the formate hydrogen lyase reaction) will be examined.

Progress: Additional information was sought regarding mechanisms whereby MNE inhibits electron transport to nitrogenase. Inhibition curves of either hydrogen evolution or acetylene reduction vs. time are nonlinear. The convex time curves indicate that there is no immediate uptake of an inhibitory concentration of MNE. A permeability barrier was suggested by greater inhibition by higher concentrations of MNE, but the curves were still nonlinear. With the use of specific reduction assay, MNE

reduction could be detected with Clostridium, but not with the other diazotrophs. It is, therefore, likely that MNE inhibition is through a mechanism other than one dependent on its reduction.

- d. Specific Objective: Determine nutritional requirements that lead to improved growth and free-living capacity to reduce acetylene for R. japonicum and related strains.

Progress: Formation of acetylene-reducing activity in a slow-growing variant of Rhizobium sp. 32H1 (cowpea-miscellany) was found to require growth on a minimal agar medium containing casamino acids supplemented with cobalt micronutrient (0.55 μg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ per liter). A combined carbon source consisting of D-gluconate and a polyol (mannitol, glycerol, or ascorbate) was also required. There was, however, no detectable cobalt stimulation of other slow-growing, acetylene-reducing rhizobial test strains.

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NEWTON, J. W. The Relationship Between Photosynthesis and Nitrogen Fixation Revisited. Presented at M. D. Kamen Symposium, LaJolla, California, Academic Press, August 27-31, 1978 (in press).

2. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

- a. Specific Objective: Complete structural investigations of Rhizobium sp. B-4384 capsular polysaccharide (CPS).

Progress: As anticipated, methylation analysis of carboxyl-reduced, Smith-degraded CPS, $[\alpha]_{\text{D}}^{20} + 140^\circ$, revealed 3-O-methyl-L-rhamnose as the only remaining nonreducing end group. The enhanced dextrorotation indicates α -anomeric configuration of L-rhamnosyl residues. Their degradative removal converted the 1,3,4-tri-O-substituted D-galacturonic acid residues into 1,3-di-O-substituted ones and further indicated that some L-rhamnosyl residues were also appended to C6 positions of (1 \rightarrow 3)-linked glucosyls. An otherwise unchanged methylation pattern indicates that only those L-rhamnosyl residues in 3-O-linkage become

biologically 3-O-methylated. Di- and trisaccharides obtained by partial hydrolysis of the CPS were characterized as 3-O- α -D-galactopyranosyluronic acid-D-mannose, $[\alpha]_D^{20} + 66^\circ$, a new disaccharide, and its homolog, O- α -D-galactopyranosyluronic acid-(1 \rightarrow 3)-D-mannopyranosyl-(1 \rightarrow 3)-D-glucose. These and earlier results suggest a highly branched decasaccharide repeat unit having basic structural similarity to the type of R. japonicum CPS produced by strains 110 and 138. All five strains of rhizobia from Acacia decurrens have similar compositions.

- b. Specific Objective: Complete structural characterization of extracellular polysaccharides from R. japonicum B-4421 and its ineffective (for nodulation) mutant B-4422.

Progress: Methylation analyses were completed for both carboxyl-reduced polysaccharides. Perdeuteriomethylation of one permitted working out the heretofore undescribed ion impact mass spectra of the peracetylated aldonitriles of 2,3- and 2,4-di- and 2-O-monomethyl L-rhamnose. No difference in methylation products was found. Furthermore, no differences could be detected either in intrinsic viscosities over the range 25-95° C or in optical rotations over the same temperature range. Lack of either structural or conformational differences leave unanswered the possible role of these polysaccharides in the infective process.

- c. Specific Objective: Continue cooperative studies with Charles F. Kettering Research Laboratory on structure of R. japonicum 3116 138 exopolysaccharide (138 PS).

Progress: Pertrideuteriomethylation and deuterio-reduction of the oligosaccharide obtained from reductive alkaline degradation of 138 PS revealed it to be a branched pentasaccharide with 4-O-methyl-D-galactosyl and D-mannosyl nonreducing end groups, a (1 \rightarrow 3)-linked-D-glucosyl residue, a 1,3,6-tri-O-substituted D-glucosyl residue and a (1 \rightarrow 3)-linked-D-galactosyl reducing group. The latter galactose residue evidently arose through reduction of galacturonic acid during the degradation. The same types of isotopic studies were done on carboxyl-deuterio-reduced PS 138 and PS 110 from stationary-phase cultures. The results confirmed earlier studies on unreduced PS 138 and on the 138 pentasaccharide. The D-galactosyl end groups of both were 4-O-methylated to the extent of ca. 50%. PS 110 had a similar structure but only 30% of the end groups bore 4-O-methyl substituents. A surprising finding in both polysaccharides, which might relate to aspects of lectin binding, was apparent occurrence of (1 \rightarrow 3)-linked-D-galactosyls in place of (1 \rightarrow 3)-linked D-galacturonopyranosyls.

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3. Physiology of Nitrogen-Fixing Blue-Green Algal and Rhizobial Symbioses with Plants (J. W. Newton)

- a. Specific Objective: To study any unique proteins elaborated during development of the Azolla-blue-green algal nitrogen-fixing symbioses.

Progress: Using plants and blue-green algae labeled with $C^{14}O_2$, we have developed a high-resolution, two-dimensional electrophoresis and radio-autographic method for study of protein constituents in the symbiotic system. Several hundred protein components have been detected. Comparisons are now being made of symbiotic and nonsymbiotic algal and plant material in search of unique protein constituents formed during elaboration of the symbiosis.

- b. Specific Objective: Isolation of blue-green algae from worldwide collection of Azolla species (obtained in cooperative work with the University of Hawaii).

Progress: Azolla mexicana, A. fuliculoides, and A. pinata species have been obtained from the University of Hawaii and all are being sterilized and propagated under aseptic conditions in preparation for algal isolation. A. mexicana, in particular, has been freed of epiphytic microorganisms and will be used first, since this strain reproduces sexually and has been reported to excrete ammonia under field conditions. Two presumptive isolates of Anabaena azollae have been obtained from two independent seedlings of Azolla mexicana. These isolates will be useful for comparative studies with the Anabaena specimen isolated from Azolla carolinaiana.

- c. Specific Objective: The mechanism by which metronidazole (MNE) inhibits N_2 fixation and H_2 production (from the formate hydrogen lyase reaction) will be examined.

Progress: With hydrogenase-negative mutants of Escherichia coli which were also transformed with Nif genes from Klebsiella pneumoniae, there would be a way to study the relationship of hydrogenase activity to Nif genetically. Chlorate-resistant (nitrate-reductase negative) mutants of E. coli were selected. Some of these were also hydrogenase (and formic hydrogen lyase)-negative. Through transfer of RP_{41} (resistance factor) and FN_{68} (fertility factor) plasmids from K. pneumoniae, several hydrogenase-negative mutants of E. coli which also contained the Nif⁺ operon were constructed. The next step would be to transfer into the hydrogenase-negative organisms defective Nif⁺ and check for evolutionary and uptake hydrogenase activity. It should be noted that hydrogenase mutants were picked up with E. coli easily, but in Klebsiella, the chlorate effect is dissociated from hydrogenase totally; i.e., there is evidently no common control point.

- d. Specific Objective: Determine nutritional requirements that lead to improved growth and free-living acetylene reductions by R. japonicum and the cowpea-miscellany strains.

Progress: When differential growth, acetylene-reducing (AR^+) activity, and antibiotic sensitivity of asymbiotic cultures were determined, a natural variation in all three categories was found among 13 Rhizobium test strains. In contrast to AR^- strains, the AR^+ strains grew uniformly and reduced acetylene consistently on a differential agar medium where 0.1% L-glutamate was the sole N source and 1% gluconate-0.3% mannitol were C sources for aerobic, surface growth. These natural variants with different

phenotypes will serve to ascertain the transfer of nitrogen-fixing genetic traits.

- e. Specific Objective: Study polymorphic forms of Anabaena azollae.

Progress: The morphological and nuclear behavior of the Anabaena akinetes (arrested cells) from Azolla caroliniana have been examined. The nuclear material within the akinete aggregates into a dense giemsa-positive structure. When the spore germinates, the nuclear material divides, separates, but remains within the spore. With time, the wall swells and from each nuclear mass a short filament of cells (homogonium?) arises. Later the spore wall ruptures and the filaments are released. This growth pattern is significant because it represents a stage in the life cycle when the multiple genomes within the alga can fuse and become redistributed later into independent cells. Such a process is very useful for future genetic manipulation.

- f. Specific Objective: Study the life cycle of Azolla mexicana to determine the fate of the algal symbiont.

Progress: Plants have been propagated from crude spore suspensions derived from decayed A. mexicana. These plants were used to obtain material for algal isolation. Several developmental stages of fern spores were identified microscopically in the crude preparations.

4. Nitrogen Contribution of Azolla spp. in Aquatic Farming Systems
(Cooperative Agreement - University of Hawaii)

- a. Specific Objective: To study Azolla management methods for use in aquatic farming systems.

Progress: Studies on the utilization of Azolla sp. in wetland agriculture in China are being made in the Kiangsi and Guangdong provinces. These studies include breeding techniques, multiplication of nursery stocks, overwintering, analysis of productivity, and comparison of Azolla species in rice paddy experiments. The techniques will be used later in field studies in Hawaii to determine their applicability under other wetland conditions.

- b. Specific Objective: Microbiology of Azolla-blue-green algae association.

Progress: Samples of Azolla and soil from Taro, watercress, and Hasu (lotus) forms in Hawaii have been obtained. Plants are being propagated prior to sterilization and growth under aseptic

conditions for isolation of blue-green algae. Several unusual blue-green algae have also been obtained from these wetland soil systems and are now under study. In addition, studies on decomposition of Azolla and microbial predators of blue-green algae in paddys are being initiated.

Publication:

LUMPKIN, T., and PLUCKNETT, D. L. 1979. Azolla: Botany, physiology, and use as a green manure. Econ. Bot. In press.

B. BIOLOGICAL AGENTS FOR PEST CONTROL

1. Insecticidal Preparations of *Bacillus thuringiensis* and Other Microbial Insect Pathogens (D. E. Johnson)

- a. Specific Objective: Determine molecular weight composition of toxic polypeptide(s) resulting from enzymatic digestion of parasporal protein from *Bacillus thuringiensis*.

Progress: The bioassay of toxic protein preparations using insect tissue in vitro has been limited in accuracy and reproducibility in the past, apparently due to extraneous enzyme activities in the crude luciferase needed for the ATP (adenosine triphosphate) bioluminescent assay. Purification of luciferase is accomplished in a two-column procedure which can be linked to provide uninterrupted flow. Column chromatography of crude luciferase, first on Sephadex G-10 and followed by separation on G-100, provides a relatively pure luciferase. Most contaminating ATP-converting enzymes are eliminated by this procedure, effectively improving accuracy and reproducibility between determinations. This improved bioassay procedure will be advantageous for the determination of toxicity among parasporal protein fragments, which requires a very sensitive assay.

- b. Specific Objective: Examine the toxic response of cultured insect tissue to *B. thuringiensis* parasporal protein by ultramicroscopy.

Progress: Cultured insect tissue was treated with enzyme-digested (activated) parasporal protein for periods of 10, 30, 60, and 120 minutes. The appearance of visible damage can be seen in isolated cells as early as 10 minutes, but the majority of cells do not respond as quickly. Electron microscopic observations of cells treated for 60 minutes revealed massive outer membrane disruption and subsequent loss of cytoplasmic constituents. The nuclear membrane swells several times its original size, but appears

to be resistant to lysis. Even in cultures treated with activated toxin for 120 minutes, however, a few cells remain resistant to toxin damage. These studies support the hypothesis that activated endotoxin protein acts as an ionophore upon gut tissue, thereby disrupting the normal distribution of cations on each side of the gut wall.

- c. Specific Objective: Investigate alternative materials for encapsulation of B. thuringiensis for protection from environmental degradation.

Progress: Starch xanthate was examined as an alternative to wood rosin for encapsulating biological materials. Unfortunately, the use of starch xanthate involves several minutes at high pH and the presence of peroxide, both of which are detrimental to spores and crystals. Potential damage to the crystal following its solubilization at high pH is a serious disadvantage to this technique.

- d. Specific Objective: Investigate form and function of newly discovered imbedded bodies in parasporal crystals of B. thuringiensis subspecies kurstaki.

Progress: It was learned that a fellow investigator in Canada had also noted the odd lumps on crystals of the kurstaki (HD-1) strain and had removed bipyramidal crystals away from the lumps by dissolving the crystals in insect gut liquid. The remaining lumps (assumed to be the embedded bodies) were found to be not insecticidal and we have ceased investigations along this line. However, transmission electron microscopy has revealed that other strains of B. thuringiensis also produce unusual parasporal bodies and we are attempting now to correlate parasporal form with insect host specificity.

Publications:

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JOHNSON D. E. Preparing Entomocidal Products with Oligosporogenic Mutants of Bacillus thuringiensis. Patent application (P.C. 6683), 1980.

SHARPE, E. S., AND R. W. DETROY. Susceptibility of Japanese Beetle Larvae to Bacillus thuringiensis: Associated Effects of Diapause, Mid-gut pH, and Milky Disease. J. Invertebr. Pathol. 34 (1979):90-91.

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C. AGRICULTURAL CHEMICALS TECHNOLOGY FOR CROP
PROTECTION AND MODIFICATION

1. Chemistry of Novel Plant Growth-Promoting Agents (Brassins)
(M. D. Grove)

- a. Specific Objective: Synthesize a biologically active analog of brassinolide from a commercially available steroid.

Progress: Stigmasterol was transformed via a six-step synthetic sequence to a mixture of tetrahydroxy lactones isomeric with 28-homobrassinolide. Bean second internode bioassay of this mixture showed evidence for both increased cell elongation and cell division.

Publication:

GROVE, M. D., G. F. SPENCER, W. K. ROHWEDDER, N. MANDAVA, J. F. WORLEY, J. D. WARTHEN, JR., G. L. STEFFENS, J. L. FLIPPEN-ANDERSON, AND J. C. COOK, JR. Brassinolide, a Plant Growth-Promoting Steroid Isolated from Brassica napus Pollen. Nature 281 (1979):216-217.

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D. TECHNOLOGIES FOR FOOD AND FEED
USES OF FIELD CROPS

1. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

- a. Specific Objective: Determine chemical and physical properties of zeins.

Progress: The water-insoluble zein was extracted from corn meal with 70% ethanol-0.5% sodium acetate and then fractionated with 95% ethanol into soluble α -zein and insoluble β -zein components. Electrophoresis in sodium dodecyl sulfate established that β -zein has very high molecular weight (MW) while α -zein consists of lower MW proteins, lowest MW being 24,000. When their disulfide bonds are cleaved by reduction, both α - and β -zeins yield polypeptide subunits with MW's 22,000 and 24,000. These subunits are separated into over 15 different polypeptides by isoelectric focusing. Zein from high-lysine corn has less of the 24,000 subunits than that from normal corn. These results establish that the solubility of zein in alcohol dependent on the degree of intermolecular disulfide crosslinks and explains many of native zeins properties.

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KURTZMAN, C. P. Systematics of the Genera Pichia, Hansenula, Issatchenkia, Lipomyces, Citeromyces, and Pachysolen. Presented at the American Society for Microbiology meeting, Los Angeles, California, May 4-8, 1979.

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2. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (T. G. Pridham)

- a. Specific Objective: Continue operation of the Agricultural Research Culture Collection (NRRL) including original and supportive research.

Progress: Microbiology staff members of the Agricultural Research Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their supportive and original research. As of January 1, 1980, the Collection maintained 70,826 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1979, the Collection distributed 2,723 strains of which 1,726 were sent to investigators in the United States and 997 were sent abroad. Of 114 strains deposited in the patent collection, 50 were from foreign sources; 181 strains were distributed to United States researchers and 253 to foreign. Twenty-five strains of bacteria and actinomycetes were identified to species for interested parties.

A review of the literature on nonlegume nitrogen fixation with special emphasis on microbiological aspects was partially completed. Microbiological methods were developed to handle cultures of the sole authentic strain available of the Actinomycetales endophyte responsible for nitrogen fixation in symbiosis with Comptonia peregrina (Sweet Fern). This will allow cultural and physiological studies to be made and will pave the way to development of selective isolation screening of other nonlegume nodules to provide additional unique germplasm.

Progress was continued on clarifying the systematics of the genera Streptomyces and Streptoverticillium through continuing accumulation of data on whole-cell hydrolyzate analyses, morphologies, electron microscopy, and antibiotic activity patterns. Studies directed to isolation and purification of DNA for determination of moles % G + C values and DNA/DNA hybridization were initiated.

Forty-five different lots of soybeans obtained from Ohio, Texas, Georgia, Arkansas, and elsewhere were examined for content of Actinomycetales. Only two lots gave evidence of internal

infestation of such forms. Aerobic, mesophilic Actinomycetales in very small numbers were detected as external infestants of 24 of the lots; 11 of the lots showed evidence of internal infestation by aerobic, mesophilic forms, again in very small numbers.

A visiting scientist from Kuwait was schooled in philosophies and methodologies concerned with Actinomycetales systematics to assist him in developing an antibiotic screening program.

An agreement was concluded with the European Patent Organization, the Agricultural Research Culture Collection (NRRL) now being recognized as an official collection in connection with European Patent strain depositions.

Some consultation was given to a representative of the Wildlife Prairie Park, Edwards, Illinois, in connection with a fly infestation problem of their Clivus Multrum organic Waste Treatment System (aerobic, waterless toilets). Some groundwork was laid for cooperative studies on microbiological monitoring of the systems for which there appears to be a need.

During the period 1966-1972, an international cooperative study was carried out (The International Streptomyces Project) on characterization of some 600 differently named taxa. The complete collection of some 3,600 lyophilized preparations is now a part of the Agricultural Research Culture Collection (NRRL) because retirement of one of the principal investigators made loss of this important collection imminent. We now maintain all remaining preparations of these standard materials.

- b. Specific Objective: Continue studies of agriculturally significant bacteria.

Initiate DNA homology studies of agriculturally significant lactobacilli. DNA from known representative strains and different isolates will be obtained in pure form and G+C contents and DNA/DNA will be determined.

Progress: DNA was extracted from 10 starch-hydrolyzing Lactobacillus strains isolated from cattle waste- and swine waste-corn fermentations, and from the type strains of L. acidophilus, L. jensenii, L. leichmanii, L. casei, and L. plantarum. The average G+C contents of the purified DNA from the cattle waste Lactobacillus and from the swine waste Lactobacillus were 40.4 and 45.5 mol %, respectively. Homology values ranging from 5 to 15% were measured between the purified DNA's from the cattle waste Lactobacillus and the type strains of L. acidophilus, L. jensenii, L. leichmanii, L. casei, L. plantarum, and the swine waste

Lactobacillus. Similarly, low homology values were found for interaction of DNA from the swine waste Lactobacillus and that of the previously mentioned type strains. Homology values of 85% or higher were found for interaction of DNA from the cattle waste Lactobacillus. Similar findings were obtained for DNA from swine waste Lactobacillus. Control experiments showed 85% or higher homology values for interaction among DNA's from four strains of L. acidophilus. Homology values among the DNA's from L. acidophilus, L. jensenii, L. leichmanii, L. casei, and L. plantarum were consistently less than 15%.

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NAKAMURA, L. K. AND C. D. CROWELL. Lactobacillus acidophilus, A New Starch Hydrolyzing Species from Swine Waste-Corn Fermentation. Dev. Ind. Microbiol. 20 531-540.

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PRIDHAM, T. G. Maintenance of Nonsporulating Molds--Speculations. Newslettr. U.S. Fed. Cult. Coll. 9(4) (1979):6-13.

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Reports:

PRIDHAM, T. G. Validation of Names of Some Streptomycetes and Streptovercillia. Final Report of Working Sub-Group

(T. G. Pridham, Chairman), International Subcommittee on Taxonomy of the Actinomycetes, 22 pp.

PRIDHAM, T. G. Culture Collections--1979. Presented at meeting of Government Patent Lawyers Association, Crystal City, Maryland, September 12, 1979.

3. Effect of Immobilization Procedure and Carrier on Enzymes That Hydrolyze Cereal Food Polymers (K. L. Smiley)

- a. Specific Objective: Immobilize both endo- and exodextranases and test their ability to degrade dextrans to unique biopolymers.

Progress: A limit dextrin was prepared from B-512 dextran by prolonged action of isomaltodextranase (G_2) prepared from Arthrobacter globiformis T6 (B-4425). The undigested portion (limit dextrin) of the B-512 dextran was then used as a substrate for other dextranases. All the dextranases tested hydrolyzed the limit dextrin with formation of a series of oligosaccharides. The oligosaccharides were identified as belonging to the straight chain isomaltose series except those formed by the Penicillium lilacinum dextranase. Co-chromatography of the oligosaccharides from P. lilacinum enzyme with authentic isomalto-oligosaccharides resulted in elution of double peaks at positions for DP-5 through 8. As yet, the unknown oligosaccharides have not been characterized. Dr. T. Sawai, a visiting scientist from Aichi Kyoiku University, Japan, prepared extended digests of B-512 (F) and B-1355 (S) dextrans using G_2 . The digests were characterized by methylation analysis of the 80% methanol insoluble fraction and by HPLC analysis of the methanol-soluble fraction. Interestingly, the methylation analysis of the G_2 limit dextran of B-1355 (S) differed significantly from the native dextran in that nonreducing end groups and branch points increased about twofold while unbranched α -1,3-linked glycosyls were much fewer. The excess of 1,6 over 1,3 linkages suggests that the limit dextran contains successive 1,6-linked residues between the 1,3-linked components. The native B-1355 dextran shows only a slight excess of 1,6 over 1,3 linkages. Apparently B-1355 dextran is structurally heterogeneous. HPLC analysis of the CH_3OH soluble fraction shows, in addition to isomaltose, a trisaccharide that elutes before isomaltotriose. In line with the properties of the G_2 enzyme as outlined by Dr. Sawai, the unknown triose is probably a terminal trisaccharide: $G-^3G-^6G$. This finding is considered highly important in view of the widespread interest in B-1355 dextran fraction S.

- b. Specific Objective: Extend studies of immobilized glucanases to α and β -1,3 glucanase(s).

Progress: Considerable time and effort was necessarily expended in preparation of substrates and enzymes required for study of 1,3-glucan degradation. α -1,3 Glucan was prepared from cell walls of Aspergillus niger, Cryptococcus terreus, C. albidus, and the tramal portion of Polyporous betulinus. 1,3 Glucanases were isolated from culture liquors of Cladosporium resinae, Rhizopus arrhizus, and a basidiomycete received from the Quartermaster collection. Cl. resinae produces both α - and β -1,3 glucanases and these were separated on columns of DEAE-Sephadex. The β -glucanase is largely exoenzymic in character although lesser amounts of endo β -1,3 glucanase may also be present. The Rh. arrhizus culture produces endo type β -glucanases attacking both β -1,3 and β -1,4 glucans. It does not produce α -1,3 glucanase. The basidiomycete QM-806 produces a β -1,3 glucanase similar to the one isolated from Cl. resinae. Both the Cl. resinae and the Rh. arrhizus enzyme complexes have been successfully immobilized on activated granular charcoal. The immobilized Cl. resinae enzyme readily attacks the β -1,3 glucan, laminarin, with formation of glucose. Gel permeation chromatography indicates no formation of intermediate-molecular-weight oligosaccharides and confirms the exoenzymic action pattern. Soluble Cl. resinae β -glucanase also shows a strong exoenzymic pattern, but small amounts of low molecular weight oligosaccharides can be detected by HPLC during the early stages of laminarin digestion. Immobilized Cl. resinae glucanase also attacks α -1,3 glucan with liberation of glucose; however, the reaction rate is very slow due to the insolubility of α -glucan under the conditions required for enzymatic activity. In order to get meaningful data on the mode of action of immobilized α -1,3 glucanase on α -1,3 glucan it will be necessary to modify the polymer to make it more soluble. The β -1,3 glucanase from Rh. arrhizus in soluble form exhibits endo-type action and a series of oligosaccharides from DP-2 to 9 can be identified by HPLC. Very little free glucose is formed. Immobilization of this enzyme, however, converts it from an endo- to an exotype with glucose being the predominant product formed.

Failure to develop a suitable assay for α -1,3 glucanase activity hinders progress in this area. No substrate levels, within bounds of solubility, can be found that limits the enzyme reaction rate. In order to look at the initial reaction rate more closely, the auto-analyzer was used to follow the reaction continuously. There was a rapid release of reducing sugar in the first minute followed by a slower steady rate over the next 10 to 20 min. This result implies that there are at least two polymers in the preparation, one of which is very susceptible to attack by the enzyme.

Increasing the substrate level increases the amount of susceptible glucan, allowing for greater production of reducing sugar with the same amount of enzyme over an equal time period.

The three β -glucanases mentioned above were tested on lichenan, a β -glucan from seaweed, and Zymosan, a commercial β -glucan prepared from cell walls of yeast. The basidiomycete and Cl. resinae enzymes that were found to have a similar pronounced action on laminarin, differed markedly on lichenan and Zymosan. The basidiomycete enzyme had high activity on Zymosan, but was only slightly active on lichenan. In contrast, the Cl. resinae enzyme reacted very strongly with lichenan but only moderately with Zymosan. The Rh. arrhizus β -glucanase showed little or no activity against Zymosan, but reacted quite strongly with lichenan. It is also very active on laminarin. Also of interest was the observation that Cl. resinae β -glucanase, which showed exoenzymic activity on laminarin, seemed to have endoenzymic activity on lichenan. All in all, these results greatly enhance the possibility of finding degradation products of β -1,3 glucans useful as agents for protection of certain plants from fungal pathogens.

Publication:

HENSLEY, D. E., K. L. SMILEY, J. A. BOUNDY, AND A. A. LAGODA. Beta-Amylase Production by Bacillus polymyxa on a Corn Steep-Starch-Salts Medium. J. Appl. Environ. Microbiol. (in press).

4. Characterization and Classification of Mucorales from Cereal Grains and Their Raw Products (C. W. Hesseltine)

- a. Specific Objective: Collect and study isolates of Rhizopus and related molds from a wide variety of sources, especially cereal grains.

Progress: During the reporting period, 89 fresh isolates were collected belonging to Rhizopus and 10 other related genera. Substrate sources were bread, cave debris, Chinese scab barley, white corn, yellow corn, numerous animal dungs and detritus, grapes, jojoba seed, papaya, patients, soybean flour, protein isolate of soybean, sunflower heads and seeds, strawberry and tempeh. These isolates will increase our reservoir for further detailed studies related to production of alcohol by Mucorales and to improved quality of food and feed.

- b. Specific Objective: Determine norm and variation in measurements of morphological characteristics for type and authentic strains of Rhizopus species.

Progress: At least 100 measurements each have been made for sporangiospore length and width and for sporangial diameter in 46 type and authentic strains of Rhizopus. Inadequate sporulation has occurred for R. niveus and examination of three homothallic species was postponed. Estimated means and standard deviations were determined. It was discovered that measurements taken at two different times gave a discrepancy of as much as 1.6 μ for the mean sporangiospore length in a strain of R. delemar. This appears to be an effect due to different nutrient conditions or, less probably, to variation in temperature. The cause of the different mean lengths is being pursued because it will probably explain discrepancies in sporangiospore measurements as reported by various authors in the literature.

- c. Specific Objective: Initially classify to species those strains labeled Rhizopus sp. in the Collection.

Progress: A total of 159 strains have been revived from lyophilized preparations and grown on appropriate media for initial classification and grouping. Of these, at least 34 strains were noted for characteristics that appear out of the ordinary for their respective species or appear intermediate to some degree between two species. They will need further detailed examination and comparison with authentic strains.

Publication:

O'DONNELL, K. L. Zygomycetes in Culture. Palfrey Contribution in Botany No. 2, 257 pp. 1979.

Report:

ELLIS, J. J. Mucorales That Can be Opportunistic Pathogens. Symposium on Taxonomy and Nomenclature of Pathogenic Fungi, American Society for Microbiology meetings, Los Angeles, California, May 1979.

5. Conversion of Cellulosic Wastes into Feed for Ruminants (P. L. 480 Grant - Pakistan Council of Scientific and Industrial Research)

- a. Specific Objective: Feeding experiments using treated wheat straw, bagasse pith, and rice straw.

Progress: Work during the last year was concerned primarily with the feeding experiments using treated wheat straw, bagasse pith, and rice straw. These cellulose were treated with calcium and sodium hydroxide, and ammonia, then fermented with one or more selected microorganisms. Dry matter digestibility with 5.5-

percent sodium hydroxide and 5-percent ammonia was 77 percent. With untreated bagasse pith, the dry matter was 27.6-percent which increased to 72.9 percent when treated with 3.5-percent sodium hydroxide. Rice straw was increased to 70.1 percent with treatment of 3.5-percent sodium hydroxide with the use of Penicillium roqueforti, and Saccharomyces cerevisiae combined with treatment of 2-percent calcium hydroxide increased the digestibility from 33.3 percent to 44 percent. All of these studies were with ruminant animals. It appears that an alkali treatment must be used before fermentation in order to get these high levels of digestibility. Some additional promising microorganisms were also isolated.

6. Fermentative Utilization of Cane Sugar Bagasses (P. L. 480 Grant - National Research Center, Cairo)

- a. Specific Objective: Isolate and identify microflora in Egyptian sugar-cane bagasse.

Progress: The work achieved on the research project included: (1) Chemical composition of seven bagasse samples; (2) chemical treatments of bagasse; (3) isolation of 169 fungal, 102 bacterial, 27 actinomycetes, and 33 yeast isolates; (4) identification of 121 fungal and 56 bacterial isolates; (5) examination of 121 fungal, 100 bacterial, and 16 actinomycetes isolates for their cellulolytic activities using culture media containing cellulose powder; (6) investigation of 61 fungal and 38 bacterial cellulose decomposing isolates for the production of single cell protein and cellulases using fermentation media containing crude bagasse as the sole carbon source.

The work achieved included the isolation from five bagasse samples, collected from the different sugar factories, of 628 microbial isolates. These comprised 249 fungal, 309 bacterial, 40 actinomycetes, and 30 yeast isolates.

Of the fungal isolates, 261, including former 48 unidentified isolates, were identified to 53 genera. One-hundred and three bacterial isolates were identified to the generic level and some to the species. These bacterial isolates belonged to 10 genera. A total of 59 actinomycetes isolates, including 19 former unidentified isolates, were identified to the generic level. All the actinomycetes isolates belonged to the genus Streptomyces.

The investigated bagasse samples varied significantly in their microflora. Variation was not only confined to the generic level but also to the species.

7. Thermophilic Microbial Conversion of Cellulosic Materials to Animal Feed (P. L. 480 Grant - Institute of Food Technology, Poznan)

- a. Specific Objective: Isolate thermophilic strains of microorganisms and elaborate simple technologies for utilization of cellulose for biosynthesis of single cell protein.

Progress: The thermophilic bacteria grown in a fermenter demonstrated a low ability to the decomposition of cellulose and dry mass in the substrate and a negligible ability to protein synthesis.

Among the six fungus cultures tested in the fermenter, the strain No. 18 demonstrated a quite good ability to the utilization of substrate dry mass and cellulose; however, its protein synthesis ability was low. The other strains tested demonstrated poor ability both to substrate decomposition and to protein biosynthesis.

The 72 strains of thermophilic fungi isolated in this study and grown by the surface technique with periodic aeration with 2:1 of air per minute every 48 hours demonstrated an ability to the utilization of wheat straw dry matter. The loss of dry matter after 8 days of growth varied from 3.0% to 25.5% and the activity of endo 1.4 β -glucanase measured by the viscometric technique varied from 0.066 to 1.15 units. The complete assessment and characteristic of these strains will be performed after quantitative calculation of substrate components used for the production of the microbial protein.

E. TECHNOLOGIES FOR FOOD AND FEED
USES - ANIMAL PRODUCTS

1. Conversion of Feedlot Wastes into Feed Supplements by Fermentation with Grain (G. R. Hrubant)

- a. Specific Objective: Demonstrate pathogen-free capability of the fermentation of cattle waste with corn by addition of enterics, mycobacteria, and viruses to the continuously operating system.

Progress: All tests were run using feedlot waste liquid (FLWL) from cattle fed a high-energy ration containing rumensin. Quantities of corn and FLWL were added at 30-minute intervals to give an average residence time of 36 hours per chamber in the three-chambered fermentor. In chambers 1, 2, and 3, pH remained essentially constant at 4.4, 4.3, and 4.2, respectively. A fecal

coliform, resistant to aureomycin and dihydrostreptomycin, was tested on fecal coliform medium with antibiotics added and on the same medium without its constituent dyes. Acid-injured cells grew only on the dye-deficient medium. Fecal coliforms, initially 10^9 organisms/g ferment in chamber 1, were killed in 4 hours by the standard method (fecal coliform medium). But 10^2 organisms/g remained as injured cells at that time. All were killed by 6 hours. Sufficient coliforms passed into the second chamber to give 10^4 organisms/g after 30 minutes. All were killed in 3 hours. No coliforms were recovered from the third chamber. One-hundred grams (wet weight) of 4-week-old Mycobacterium paratuberculosis cells, muddled--not blended--in FLWL, were added to chamber 1. After 5 minutes, 10^6 colony-forming units were recovered per gram of ferment. After 6 hours, one colony-forming unit per gram ferment was recovered. No M. paratuberculosis was recovered from chamber one after 1 day. No M. paratuberculosis was recovered from chambers 2 or 3. Five bacteriophage, selected for shape and nucleic acid composition, were used to test virus kill. Each was added to give 10^9 viruses/g ferment in chamber one. Ø6--2-RNA, polyhedral with lipid envelop--was killed within the 5-minute mixing time required to uniformly distribute the inoculum in chamber one. The four remaining phage were carried through the fermentor so that after 12 hours up to 10^6 organisms/g were recovered in the output. The killing curves were essentially hyperbolic; and, after 1 day, curves for chambers one and two and for output were superimposable. ØX174--1-DNA, icosohedral with large apical capsomeres--and PL-1--2-DNA, icosohedral head with long tail--were reduced in numbers to 10^2 viruses per gram in 2 days. From 0-70 viruses/g ferment were found thereafter up to 5 days (experiment terminated). Z1K/1--1-RNA; polyhedral with uniform capsomeres--and ZJ/2--1-RNA, filamentous--were reduced in numbers to 10^4 viruses/g in 3 days. From 10^4 - 10^3 viruses/g were found thereafter up to 5 days (experiment terminated).

- b. Specific Objective: Initiate studies to examine the interrelationship of the principal organisms, Lactobacillus, coliform bacteria, and yeasts involved in the swine waste-corn fermentation.

Progress: Organisms used in the studies were homofermentative strach-hydrolyzing Lactobacillus, heterofermentative Lactobacillus, Candida krusei, and fecal coliform bacteria isolated from pig waste-corn fermentations. When cultured together in laboratory media, the heterofermentative Lactobacillus appeared to hasten the death of the homofermentative Lactobacillus in the stationary growth phase. Although the initial multiplication of fecal coliform bacteria was not inhibited by the presence of the homo- or heterofermentative Lactobacillus or

mixtures of both, rapid diminution of fecal coliform bacteria numbers was observed when the Lactobacillus achieved maximum growth and caused a steady decrease of the pH to a value of about 4.0. In single cultures, fecal coliform bacteria achieved population densities of about 10^9 /ml. in 24 hours and maintained this number for at least 72 additional hours. In mixed culture with Lactobacillus, however, fecal coliform numbers diminished from 10^9 cells/ml. at 24 hours to approximately 5×10^4 cells/ml. at 96 hours. Although extended incubation caused slow decrease of numbers, survivors were detected after 168 hours. The adverse effects of Lactobacillus towards fecal coliform bacteria appeared to be diminished by the presence of a yeast (Candida krusei). For example, the reduction of fecal coliform bacterial numbers was smaller in mixed cultures consisting of yeast, Lactobacillus, and fecal coliform bacteria than in those containing fecal coliform bacteria and Lactobacillus. In the mixed cultures containing yeast, the pH values reached 4.6 at 24 hours but then increased to 5.2 after 96 hours. When incubation of these cultures was extended to 168 hours, the pH value increased to 5.8 and fecal coliform bacteria increased in numbers about 100-fold. Evidence indicated that the reversal of the pH decrease was caused by the yeast consuming the organic acid produced by the Lactobacillus.

- c. Specific Objective: Continue study of semicontinuous batch fermentation of fresh swine waste combined with corn. Examine effect of increased time on batch fermentation or of adding whey to kill coliforms during the fermentation.

Progress: Ten-percent portions of fermentation product from fresh swine waste-corn cultures were used serially as inocula at 28° C. for three separate feedback intervals of 48, 772, and 96 hours. Consistent cyclic kill of coliforms in all feedback intervals with pH levels near 4 did not take place. Coliform kill in low acid content cycles (pH 6.1) was also recorded but coliforms were present after these instances of disappearance with pH levels again near 4. A search for antibacterial substances elaborated by the dominant group of lactic acid bacteria showed methanol and acetone extracts of selected end of cycle samples inhibited coliform bacteria isolated from pig waste. These isolates were also inhibited by the homologous series of fatty acids from formic through valeric acid and lactic acid but the extraction protocol for antibacterial substances involved lyophilization which removes volatile fatty acids leaving lactic acid. A separation was effected between an antifecal coliform substance with an R_f value of 0.36 and lactic acid. Data suggests that short-chain fatty acids suppresses coliform bacteria but cannot effect repeated cyclic coliform kill without the presence of antibacterial substances. Separation of quantities of antibacterial matter by column chromatography has not been successful.

Publication:

GOLUEKE, C. G., W. J. OSWALD, AND B. A. WEINER. Waste Treatment and Utilization. In Microbial Processes: Promising Technologies for Developing Countries, Chap. 7, pp. 124-141, National Academy of Sciences, Washington, D.C. 1979.

F. TECHNOLOGIES FOR INDUSTRIAL USES
OF PLANT AND ANIMAL PRODUCTS

1. Increased Energy Efficiency of Substrate Preparation for Alcohol Fermentations (R. W. Detroy)

- a. Specific Objective: Investigate new chemical processes to more efficiently convert plant polysaccharides to fermentable sugars and recover the nutrients from fermentation byproducts to decrease energy requirements for alcohol production.

Progress: Wheat straw (WS) was pretreated separately with various concentrations of anhydrous NH_3 , NH_4OH , NaOH , HCl , H_2SO_4 , dimethylsulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), and ethylenediamine (EDA), followed by treatment with cellulase. Chemically treated WS also was disc-milled prior to the addition of enzyme. Conversion amounts of cellulose to sugar varied with chemical treatment. Conversion of the cellulose to sugar of the NH_3 - and EDA-treated WS was 35 and 60%, respectively, compared to 11% for the untreated WS. EDA-treated WS showed a substantial decrease in lignin compared to all other WS treatments. Disc-milling of chemically treated WS residues resulted in 72 and 83% conversion of the NH_3 - and the EDA-pretreated WS compared to 17% in milled control straw. About 40% cellulose was converted in acid-treated straw, and 55% in alkali-treated straw. Scanning electron photomicrographs of wheat straw demonstrate structural changes that occur when the straw is treated with alkali, acid or EDA, followed by enzymatic (cellulose) treatment; apparently, chemical pretreatment permits ready enzymatic digestion of the pithwall and destruction of many parenchyma cells. Such treatment resulted in loss of about half of the original straw stem mass. EDA-treated milled straw followed by enzymatic hydrolysis results in an amorphous mass of plant tissue, probably mostly epidermal, plus some strengthening tissue of the straw.

- b. Specific Objective: Investigate the ability of various microorganisms to more efficiently convert lignin to fermentable compounds and feedstock chemicals.

Progress: The oyster mushroom fungus Pleurotus ostreatus NRRL 2366 was grown on wheat straw (WS), and the fermentation products were analyzed for degradation of lignin and for resulting cellulose components. The fungus-fermented WS was subjected to cellulase treatment, and saccharification values were obtained from subsequent residue hydrolysis. After 10 and 20 days' fermentation, the enzymatic conversion of remaining cellulose to glucose was 15 and 17%, respectively, compared with 15% in control straw. However, after 30, 40, and 50 days' fermentation, the glucose conversion was 34, 56, and 72%, respectively.

Differentially ^{14}C -labeled, naturally complexed lignocelluloses (LC) were employed to evaluate specific fungi for degradation and solubilization of lignin and for cellulose hydrolysis. Three basidiomycetous fungi, Pleurotus ostreatus, Phanerochate chrysosporium, and an unidentified Michigan basidiomycete were grown on ^{14}C -lignin (L*C) or ^{14}C -glucan (LC*) substrates for 19 days at 26 C. Lignin biodegradation was assayed by the conversion of L*C residue to $^{14}\text{CO}_2$ and other ^{14}C -lignin H_2O soluble components, whereas cellulose hydrolysis was measured by conversion of the (LC*) residue to $^{14}\text{CO}_2$.

Nutrient N levels were investigated to determine whether these fungi would preferentially degrade the ^{14}C -labeled lignin or glucan-LC. At 20 mM N, lignin degradation was suppressed 15 and 60%, respectively, for P. ostreatus and P. chrysosporium after 19 days' growth. The Michigan basidiomycete was unaffected by the N levels. P. ostreatus and P. chrysosporium degraded 20% of (L*C) component, whereas the Michigan basidiomycete converted 40% of the ^{14}C -lignin component to $^{14}\text{CO}_2$ at 2 mM N or less.

Cyathus stercoreus (Schw.) de Toni, NRRL 6473, isolated from aged and fragmented cattle dung collected from a Michigan pasture, effected substantial losses in lignin (45%) from wheat straw (WS) during a 62 day fermentation (25°C). The basidiomycete also improved WS digestibility by freeing α -cellulose for enzymatic hydrolysis to glucose (230 mg glucose/1,000 mg fermented residue). The rationale for selecting C. stercoreus in attempting to biologically modify the lignin and cellulose components in WS or other graminaceous agricultural residues was based on the expectation that this organism is ecologically specialized to enzymatically attack the substructures of native lignins in grasses.

- c. Specific Objective: Evaluate liquification of raw or modified starch by testing in a batch process (1) several amylolytic microorganisms in situ and (2) enzymes separated and harvested from these same microorganisms.

Progress: Preliminary investigations indicate that Bacillus subtilis NRRL 3696, B. macerans NRRL 430, Aspergillus oryzae NRRL 468, A. awamori NRRL 3112 and A. foetidus NRRL 337 all grow on untreated cracked corn when incubated at 28°C for three or more days. The two bacteria grew poorly whereas the fungi grew very well. About 3-10% of the untreated cracked corn is converted to glucose within three days incubation; after this period both the bacteria and fungi utilize about 90% of the glucose for cellular reproduction. The bacterium Bacillus macerans and the fungus Aspergillus foetidus seem to be the most promising organisms for production of glucose from untreated corn starch.

Preliminary experiments also involved the inoculation of water soaked cracked corn with the fungi Aspergillus foetidus 337 and the yeast Saccharomyces cerevisiae NRRL Y-2034 and incubating at 28°C for several days at which time analysing for both glucose and alcohol production. Results indicate that alcohol can be produced from crudely cracked corn in this manner but more experiments are needed to determine proper inoculum size and ideal incubation time. In these preliminary experiments about 10% corn was converted to glucose and subsequently about 0.2% alcohol produced.

Promising research began on the hydrolysis of corn starch with Amylomyces rouxii. Amylomyces rouxii, a unique fungus isolated from fermented foods in Indonesia, reduced the starch content in 20 l batch fermentations from 9 to 3.5% and produced 3% glucose and 1% ethanol. Through subsequent research with this fungus coupled to other microorganisms, the energy efficiency of ethanol production may be improved.

- d. Specific Objective: Mutate Phanerochaete chrysosporium for loss of cellulase activity without loss of ability to degrade lignin.

Progress: P. c. NRRL 6422 was selected for mutation work on the basis of maximum ratio of mono- to multinucleate spores of the 18 strains available. Ultraviolet light treatment produced many presumptive mutants by the cellulose clearing technique but all produced enough cellulase to release dye from cellulose azure under the conditions used. Nitroso guanidine produced many presumptive (Cel-) mutants on cellulose plates. One such colony failed to release dye from cellulose azure. This mutant, when compared to its parent, grows more slowly on malt extract agar plates and sporulates sparsely and spottily. When these abnormal spores are subjected further to nitroso guanidine, they are more easily killed than the spores of the parent.

- e. Specific Objective: Evaluate botanochemical fiber-residues in papermaking and other applications.

Progress: Guayule soda pulp has been further evaluated for papermaking. During chemical analysis, the guayule alpha cellulose contained an inordinate amount of pentose sugar. Guayule fibers, which resemble hardwood fiber in shape, are short (0.3 mm, average) and form paper sheets of low strength. Blends containing up to 70% guayule pulp admixed with bleach softwood kraft formed papers satisfactory for several purposes.

Publications:

LINDENFELSER, L. A., R. W. DETROY, J. M. RAMSTACK, AND K. A. WORDEN. Biological Modification of the Lignin and Cellulose Components of Wheat Straw by Pleurotus ostreatus. Development in Industrial Microbiology, the Society for Industrial Microbiology, Vol. 20, 1979.

DETROY, R. W. AND C. W. HESSELTINE. Availability and Utilization of Agricultural and Agro-Industrial Wastes, Process Biochemistry, Vol. 13, No. 9, 1978.

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DETROY, R. W. Biological Delignification of ¹⁴C-labeled Lignocelluloses by Basidiomycetous Fungi: Degradation and Solubilization of the Lignin and Cellulose Components. ASM Abstract. Miami Beach, Florida, May 10-15, 1980.

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Reports:

DETROY, R. W. AND G. ST. JULIAN. Exhibition of NRRC Research on Alternative Energy Resources. Springfield, Illinois, Farm Energy Exposition. November 2, 1979.

DETROY, R. W., R. EISENHAUER, J. MCGHEE, AND G. ST. JULIAN. Iowa Energy Fuels Expo, Exhibition of NRRC Research. December 11-12, 1979.

2. Innovative Fermentation Technology for Alcohol Production
(R. W. Detroy)

See Biomaterials Conversion Laboratory, B. 9.

G. TECHNOLOGIES AND PRODUCTS TO INCREASE
EXPORTS OF AGRICULTURAL PRODUCTS

1. Soybean Foods of the Traditional Oriental Type for the Export Markets
(H. L. Wang)

- a. Specific Objective: Investigate in-situ heat-resistant bacterial spores in soybeans.

Progress: A total of 65 samples representing the 1978 crop was collected from North Central Illinois, Northeastern Ohio, Western Texas, Georgia and Arkansas. Internal bacterial contamination averaged from 4 to 26%, the highest being in the samples from Arkansas, Iowa and Illinois with 26%, 23%, and 24% respectively. Georgia showed an average of 15% internal contamination. Ohio and Texas had the least amount of contamination with 5% and 4% respectively.

Seventy-eight isolates obtained from the above samples have been taxonomically studied. Sixty of them were spore-forming and belong to 12 species of Bacillus: B. lacterosporus, B. licheniformis, B. macerans, B. brevis, B. pumilus, B. megaterium, B. subtilis, B. sphaericus, B. larvae, B. coagulans, B. polymyxa, B. cereus, in the order of their declining occurrence. Spores produced by each of the 12 species were tested for their heat resistance. Spores produced by B. licheniformis, B. brevis, B. subtilis, B. coagulans, and B. cereus survived after 90 min. at 100°C, spores of B. megaterium and B. larvae survived after 30 min. at 100°C, but were inactivated after 40 min. and 70 min. at 100°C respectively. Spores of the most frequently found species, B. laterosporus, and the other three species were inactivated after 30 min. at 100°C.

- b. Specific Objective: Investigate the influence of coagulative conditions on the quality and yield of tofu.

Progress: Tofu, a cheese-like product, is made from soybean milk by coagulating the protein with calcium or magnesium salts. Protein denaturation, by boiling the soybean milk before the addition of salt, was found necessary to form the desired structure of the aggregates. Ionic strength of the salt affected the curd yield as well as the curd texture and the curd volume.

At a very low concentration of calcium chloride (0.002 M), there was no precipitation or curd formation. As the salt concentration increased from 0.006 M to 0.1 M, the tofu-gel became firmer and the total solid recovery was increased; the supernatants were clear. However, when the salt concentration was further increased, the supernatant became turbid and the gel was imperfect. At 0.4 M of calcium chloride, there was no gel formation, although precipitation was observed. It seemed that at high concentration of salt, the water-holding capacity of the protein was very low.

- c. Specific Objective: Evaluate U.S. soybeans to identify the most suitable varieties for miso fermentation.

Progress: Miso, a paste-like product prepared from soybeans and rice with Aspergillus oryzae, Saccharomyces rouxii and Pediococcus halophilus, is one of the most important fermented soybean products in Japan. The annual production in Japan amounts to more than one billion pounds. Currently, the Japanese miso industry imports most of their soybeans from China. They believe Chinese beans are superior to those from the U.S. for making miso. This project, with the financial support from the Dekalb County Farm Bureau, Dekalb, Illinois, was to evaluate for miso fermentation the common varieties of soybeans now produced in the U.S.

Thirty-six samples of 16 American varieties, 4 Japanese varieties and 10 Chinese varieties were examined with respect to moisture; weight of 100 beans; content of protein, oil and carbohydrates; weight increase after soaking and cooking; and color and tenderness after cooking. Organo-leptic tests of miso made from 7 selected U.S. varieties were carried out in Japan. Our data show:

1. Protein content of U.S., Chinese and Japanese beans is very similar and averaged 40.8%, 40.7% and 41.3% respectively.
2. U.S. soybeans have higher oil content than Chinese or Japanese beans, 20.6%, 18.5% and 17.3% respectively.
3. Japanese beans have the highest carbohydrate content, 22.0%, followed by 20.7% for Chinese beans and 19.3% for U.S. beans.
4. All U.S. beans, except MI brand, are smaller than Japanese beans. The Chinese beans are the smallest. The weights of 100 beans from U.S., Chinese and Japanese samples averaged 21.6 g, 16.1 g and 32.1 g respectively.

5. Japanese and Chinese soybeans absorb and hold water during cooking slightly better than U.S. beans, except MI brand.
6. Tenderness of cooked soybeans varied greatly between all samples.
7. It has been said that Japanese soybeans have a high carbohydrate content, consequently they absorb water well and cook to softness. No clear co-relationship was found based on our data. It is difficult to confirm the direct relationships between carbohydrate content and water content after soaking and cooking, or that the carbohydrate affects tenderness.
8. Based on the qualities of the beans most similar to those of Japanese beans, 7 U.S. varieties (Harcor, Corsoy, Hark, Peterson 3105, McCoy 1100, MI-brand and Amsoy) were selected to make miso. Organoleptically, the differences between them were small. Miso made from McCoy 1100 had an outstanding color and that made from MI-brand had a good consistency.

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HESSELTINE, C. W. Fermented Soybean Research at the Northern Regional Research Center-Past and Present. Presented at Soycraft Conference, Amherst, Massachusetts, July 26-29, 1979.

WANG, H. L. Research at the Northern Regional Research Center on Oriental Fermented Foods. Presented at the International Symposium on Oriental Fermented Foods. Taipei, Taiwan, Dec. 9-15, 1979.

H. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Studies on Mycotoxins in Cereal Grains and Their Control (C. W. Hesseltine and O. L. Shotwell)

- a. Specific Objective: Determine conditions required for destruction of Fusarium toxins, particularly zearalenone and deoxynivalenol, in ground and whole kernel corn and reaction products formed during destruction.

Progress: Reagents ammonium hydroxide or formaldehyde destroyed zearalenone in contaminated ground corn, but were not effective in reducing toxin levels in whole kernel corn.

- b. Specific Objective: Develop method for treating solid products from alcoholic fermentation of contaminated corn to destroy or remove Fusarium toxins.

Progress: Ethanol fermentations were carried out using field-inoculated corn obtained in cooperation with the University of Illinois that contained 33.5 ppm zearalenone. Formaldehyde destroys zearalenone in solids recovered from fermentations. Ammonium hydroxide and sodium hydroxide were less effective in detoxifying contaminated solid products from the fermentation probably because of the high oil content in the products.

- c. Specific Objective: Develop analytical methods for Fusaria toxins, particularly zearalenone, deoxynivalenol, diacetoxyscirpenol, and T-2 toxin.

Progress: A rapid detection method for zearalenone is near completion that can be applied to the qualitative or quantitative analysis of corn. Extracts are purified by a zinc acetate-sodium chloride treatment followed by a liquid-liquid partition. Zearalenone is detected and measured in purified extracts by thin-layer chromatography (TLC) and by high-pressure liquid chromatography (HPLC). Preliminary experiments indicate that reverse-phase HPLC can be used to detect and measure deoxynivalenol in partially purified extracts from corn. The toxin can be separated from interfering material with water-methanol (90+10) and can be detected with a UV detector at 224 nm. Studies comparing HPLC and TLC measurements of zearalenone in corn with the National Institute of Public Health, Bilthoven, The Netherlands, showed that recoveries were $81.9 \pm 10.1\%$ by TLC and $76.1 \pm 7.6\%$ by HPLC. Coefficients of variation were 12.4% by TLC and 10.1% by HPLC.

- d. Specific Objective: Test effectiveness of HPLC in measuring aflatoxins in extracts of tissues, milk, and cereal grains.

Progress: Aflatoxin M_1 was measured successfully in extracts of naturally contaminated powdered milk at levels >0.025 ppb. Standard aflatoxins B_1 , B_2 , G_1 , and G_2 and aflatoxins in naturally contaminated corn were measured by HPLC equipped with a silica gel-packed cell in a fluorescent detector. Standard B_1 could be detected at a level of 10 pg. In corn extracts, 10-20 pg B_1 could be detected. Extracts of corn for HPLC could be obtained by either the CB method or by the Stubblefield-Shotwell method, but extracts obtained by the Pons methods had low recoveries (54%).

- e. Specific Objective: Complete the development of assay methods for aflatoxin in liver and muscle tissues.

Progress: A method for the determination of aflatoxins B_1 and M_1 in liver has been developed for detection and determination by two-dimensional TLC and densitometry. The method has been studied collaboratively. A rapid TLC confirmation of B_1 and M_1 in liver has been developed.

- f. Specific Objective: Continue survey of wheat and corn in Virginia for zearalenone, aflatoxin, and ochratoxin. Survey 1979 corn from Georgia, North Carolina, Virginia, and Texas for aflatoxin.

Progress: None of the 93 Virginia wheat samples collected from the 1979 crop by the Federal Grain Inspection Service (FGIS) had detectable zearalenone, aflatoxin, or ochratoxin A. The FGIS collected 114 1979 corn samples from Virginia, none of which had zearalenone or ochratoxin A. Out of the 114 corn samples, 33 had detectable aflatoxin; 13 had <20 ppb; 8 had 20-100 ppb, and 12 had 100-500 ppb. Of 84 1979 Georgia samples collected by the Statistical Reporting Service (SRS) (USDA), 67% had detectable aflatoxin; 31% had >20 ppb; and 13% had >100 ppb (highest was 1,637 ppb). Of 108 1979 Virginia samples collected by SRS, 17% had detectable aflatoxin; 11% had >20 ppb; and 5% had >100 ppb (highest was 465 ppb). Of 109 1979 Texas samples, 13% had detectable aflatoxin and 6% had 20-100 ppb.

- g. Specific Objective: Study enhancement of aflatoxin levels in samples after collection.

Progress: Fifty-seven sets of two equivalent corn samples (from the same location in a given field) were collected in Georgia representing the 1979 crop. One sample of each set was dried immediately in Georgia before shipping to Peoria for analysis; the other sample was shipped and then dried in Peoria before analysis. Samples were in transit 4-7 days and longer. Of the samples dried in Georgia before shipping, 37% had detectable aflatoxin, and 18% had >20 ppb aflatoxin. Of those that were not dried before shipping, 63% had detectable aflatoxin and 28% had >20 ppb. There was definitely toxin formation during shipping. Eight samples from Virginia and Texas were not completely dried in Peoria ovens before analysis and went out of condition resulting in aflatoxin level of 520 ppb.

- h. Specific Objective: Develop a bioassay to determine the relative refusal rate of Fusarium metabolites.

Progress: The acceptance or rejection by mice of Fusarium toxins, a test to be used for the detection of refusal factors, particularly the trichothecenes, in feeds or culture extracts has been studied. Of the 10 purified Fusarium metabolites studied, only the trichothecenes--diacetoxyscirpenol, T-2 toxin, and vomitoxin--were refused. The acceptance levels of trichothecenes by mice were about the same as those reported for swine.

- i. Specific Objective: Screen the Fusarium cultures in the Agricultural Research Culture Collection (NRRL) for metabolites that are potential refusal factors for swine.

Progress: Fusarium cultures (132) were screened for refusal factor production potential. Extracts from about 35 cultures were

refused by mice. These cultures will be retested and specific refusal factors identified.

- j. Specific Objective: Study production of trichothecene mycotoxins.

Progress: Several T-2 toxin-producing Fusarium strains were grown and T-2 toxin production measured. The combination of a good toxin-producing strain and cultural conditions conducive for introduction of ^{14}C labeling has not yet been found. Extracts from cultures of Fusarium nivale (NRRL 3509) were screened using a mouse bioassay and a trichothecene-specific thin-layer chromatographic spray to correlate toxicity with appearance of a blue color. The presence of the tetrahydroxy trichothecene nivalenol in one purified fraction was detected by combined gas chromatography-mass spectrometry.

- k. Specific Objective: Cooperate with the University of Illinois, College of Veterinary Medicine, to determine the fate of T-2 toxin consumed by swine.

Progress: Crystalline T-2 toxin was produced for use in its determination in feeds, blood plasma, and urine. The development of sample cleanup procedures coupled with gas chromatographic analysis permits detection of less than 5 ppm T-2 toxin in feed and less than 20 ppb in blood plasma and urine.

- l. Specific Objective: Produce and purify gram quantities of xanthomegnin for animal toxicity studies.

Progress: Static culture fermentation of Penicillium viridicatum (NRRL 6430) was investigated and optimized for the elaboration of xanthomegnin. Yields in excess of 1 g/kg were obtained from rice fermented at 15°C. A novel solvent/solvent partition of the crude methylene chloride extract provided a relatively clean xanthomegnin product. Final purification by preparative high performance liquid chromatography was developed to afford a multi-gram quantity of crystalline xanthomegnin. Identity of the compound was confirmed by nuclear magnetic resonance and mass spectrometry. A sample was furnished Dr. W. Carlton, Purdue University, Department of Veterinary Microbiology and Pathology for toxicopathological studies in rodents, chickens, and swine.

- m. Specific Objective: Continue structural studies on secondary metabolites synthesized by molds of the genera Penicillium, Fusarium, and other common fungi from cereals.

Progress: The structure of the antibiotic equisetin has been determined to be a new 3-acyl N-methyl tetramic acid. A metabolite from Penicillium egyptiacum was identified as the isonitrile antibiotic xanthocillin.

- n. Specific Objective: Assess toxicoses of farm animals thought to be caused by consumption of moldy grains or feeds made from infected grains.

Progress: The trichothecene, vomitoxin, was found in two Austrian corn samples at levels of 1.3 and 7.9 ppm and in one Canadian corn sample at a level of 7.9 ppm; all three samples were rejected when fed to swine. Feed made from the latter corn sample contained 1.4 ppm vomitoxin. Kernels from these corn samples were examined for Fusarium graminearum. The mold was found only in the two Austrian corn samples and four of the isolates produced vomitoxin ranging from 3.7 to 16.7 ppm.

- o. Specific Objective: Study mycotoxicoses in equine in cooperation with the University of Illinois, College of Veterinary Medicine.

Progress: Signs and lesions characteristic of classical equine leucoencephalomalacia were produced in one of two donkeys given corn cultured with Fusarium moniliforme. Gross and histopathological lesions of the cerebrum included an extensive necrotic cavitation within one cerebral hemisphere, disruption, and rarefaction of the subcortical white matter and prominent perivascular cuffing. Three rabbits fed the cultured corn did not develop characteristic signs or lesions of the toxicosis. Extracts of the cultured corn were nontoxic to 1-day-old chicks.

- p. Specific Objective: Elucidate reaction pathways involved in the destruction of aflatoxin-like model compounds during ammoniation of corn.

Progress: A model ketocoumarin was adsorbed onto an inert media and ammoniated to give a large number of products, two of which have been isolated and characterized. The first, a phenol, is a transitory intermediate capable of undergoing further reaction. The second possesses an unexpected enolized alpha-diketone structure.

- q. Specific Objective: Describe the microfungus community associated with a rodent seed cache economy millions of years old and determine the mycotoxin-producing capabilities of species known to produce toxins in cereal agroecosystems.

Progress: Species populations of molds associated with different stages in seed harvesting and seed storage by a heteromyid rodent from the Sonoran Desert (kangaroo rat) were described and quantified. Among the prevalent molds isolated were several known mycotoxin producers (i.e., Aspergillus flavus, A. ostianus, A. ochraceus, A. versicolor, A. nidulans, A. rugulosus, Penicillium griseofulvum, P. cyclopium). All of the A. flavus strains examined to date produced aflatoxin in laboratory fermentations of cracked corn. Isolates of A.

versicolor, A. nidulans, and A. rugulosus produced sterigmatocystin during fermentation on cracked corn. However, strains of A. ochraceus and A. ostianus tested failed to produce ochratoxin or penicillic acid under similar conditions. Because kangaroo rats contaminate the seeds they gather with toxigenic fungi, the strategies heteromyids have adapted for seed handling may preclude the toxins from being an important aspect of the natural system. These strategies are currently being investigated.

- r. Specific Objective: Determine levels of aflatoxin and ochratoxin in sclerotia versus conidia of toxigenic strains of A. flavus and A. ochraceus group.

Progress: Preliminary studies have shown that sclerotia of A. flavus and A. parasiticus do not contain substantial quantities of aflatoxin as contrasted with equivalent weights of dried mycelium and culture media from the same culture. Work with A. ochraceus group is now underway.

- s. Specific Objective: Examine the discriminating value of ultraviolet-induced fluorescence in APA test medium when distinguishing among aflatoxin-producing and nonproducing strains of A. flavus isolated from cereals.

Progress: Randomly selected isolates of A. flavus from corn grown in North Carolina were tested for their ability to produce (1) a blue fluorescence on APA test medium; (2) aflatoxin in sterilized cracked corn. All of the strains producing a blue fluorescence on APA test medium produced substantial quantities of aflatoxin when grown on cracked corn. Two of 12 APA-negative strains produced large quantities of aflatoxin on cracked corn. Single conidium isolates of these strains were both aflatoxin-positive and aflatoxin-negative. Because the original A. flavus isolates tested were taken from cultures grown out from individual corn kernels and not single spores, genetic heterogeneity probably accounts for the test results described above.

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2. Products of Reactions Between Mycotoxins and Food and Feed Constituents from Corn (C. W. Hesseltine)

- a. Specific Objective: Study the chemistry of patulin to determine the molecular basis of toxicity.

Progress: A total of 13 derivatives of patulin, which retain the unsaturated lactone but lack the reactive hemiacetal moiety, have been prepared for toxicological evaluation. In addition, antimicrobial disk assays were carried out using Gram-positive and Gram-negative bacteria, two yeasts, and a mold. Antifungal activity was doubled when patulin was esterified. The semi-carbazone derivative was unique in showing significant antibacterial activity against the Gram-negative Escherichia coli.

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3. Germ Plasm Bank of Microorganisms for Research on Microbial Toxins (T. G. Pridham)

- a. Specific Objective: Continue operation of the Agricultural Research Culture Collection (NRRL) including original and supportive research.

Progress: Mycology staff members of the Agricultural Research Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1980, the Collection maintained 70,826 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1979, the Collection distributed 2,723 strains of which 1,726 were sent to investigators in the United States and 997 were sent abroad. Of 114 strains deposited in the patent collection, 50 were from foreign sources; 181 patent strains were distributed to United States researchers and 253 to foreign. Four-hundred and fifty strains of molds and yeasts were identified to species for interested parties.

A total of 233 strains of molds that had been stored at 3°C for 2 years as agar plugs permeated with hyphae submerged in sterilized mineral oil were evaluated. At the end of 2 years' storage, 168 or 72% of the strains stored as agar plugs permeated with hyphae were viable whereas 150 or 64% of the strains stored as agar slants were viable. It appears, therefore, that storage of strains under sterilized mineral oil as agar plugs permeated with hyphae can be a good and convenient method of storage. The technique's advantage is that it overcomes a drying out problem that can occur with agar slant cultures overlaid with mineral oil when the tip of the agar slope becomes exposed to air. A total of 30 cultures of 21 species of fungi representing 13 genera were provided to four members of the Energy Center. Members of the Culture Collection helped select many of these thermophilic and thermotolerant fungi for their degradative abilities or their alcohol-producing potential. New isolates of *Fusaria* from feeds and known or possible trichothecene-producing *Fusaria* were selected and supplied to Mycotoxin Microbiology and Biochemistry Research for study of the swine-refusal factor. Taxonomy of such *Fusaria* was clarified.

Antimicrobial activity of patulin and 13 derivatives was determined by several methods. A number of the compounds were shown to exhibit antibacterial and/or antifungal activity.

Using ecological rationale, we selected and isolated *Cyathus stercoreus* (Schw.) de Toni, NRRL 6473, a colonist of aged and fragmented cattle dung to be used in the biological modification of lignocellulose in wheat straw and other graminaceous plant residues. Natural lignins of grasses differ in the relative frequency of certain chemical substructures from other plant taxa and *C. stercoreus* is ecologically specialized to utilize the lignocellulose in ruminant dung from grasslands. No other fungus thus far tested is as efficient in selectively attacking lignin in wheat straw (45% degraded) while, at the same time, degrading only

20% of the cellulose and freeing cellulose for enzymatic hydrolysis to glucose (230 mg glucose/ 1,000 mg fermented residue). This represents a fivefold increase in the amount of cellulose hydrolyzed to glucose as compared with an unfermented wheat straw control.

- b. Specific Objective: Study the relationship between molds from an ancient seed caching economy and those from present-day cereal agroecosystems.

Progress: Mycotoxigenic fungi traditionally associated with cereal agroecosystems (<10,000 years old) were among the prevalent molds recorded in mycological studies of an ancient rodent seed cache economy (circa mid-Miocene). Isolated from the external cheek pouches and subterranean seed caches of the Sonoran desert kangaroo rat (Dipodomys merriami) were these important mycotoxin producers: Aspergillus flavus, A. ostianus, A. ochraceus, A. versicolor, A. nidulans, A. rugulosus, Penicillium griseofulvum, P. cyclopium. We are examining these isolates for morphological characteristics that might distinguish them from strains isolated from cereal crops. Our assumption is that the strains presently contaminating cereals may have originated from earlier seed-caching economies.

- c. Specific Objective: Prepare a compendium of methods for the preservation of microorganisms, cell lines, and viruses.

Progress: Coeditors were selected, chapter titles decided upon, and preliminary contact made with potential contributors.

- d. Specific Objective: Prepare monographs on the genera Hansenula, Pichia, and Issatchenkia for the book The Yeasts, A Taxonomic Study.

Progress: Monographs of the genera Hansenula and Issatchenkia were completed and data for the treatment of Pichia was compiled.

During 1979, 32 yeast strains were provided to the Northern Agricultural Energy Center for studies on ethanol fermentation and utilization of xylose from lignin.

- e. Specific Objective: Study the occurrence of bacteria, molds, and actinomycetes on and in seeds of various crop and related plants.

Progress: About 100 samples of soybean seeds, jojoba seeds, sunflower seeds, and nodules and seeds of nonleguminous nitrogen-fixing plants were assayed microbiologically. In general, there

were very few Actinomycetales on surfaces or within the test materials. Mold and bacterial counts ranged from very low to very high. Many of the samples showed evidence of internal infestation to varying degrees.

- f. Specific Objective: Accession the Quartermaster Collection containing many cellulose degraders and molds important to degradation of materials and any other major collections in danger of being lost.

Progress: The Quartermaster Collection containing 10,188 assigned numbers and records pertinent to the Collection was transferred to NRRC. The stock consists of approximately four lyophilized preparations for each numbered strain that was maintained in lyophil and the remainder as agar slant cultures overlaid with sterilized mineral oil. The Collection consists mostly of Fungi Imperfecti, many from the Tropics and subtropics, and includes numerous strains noted for their ability to deteriorate cellulose and other materials. Some strains from this collection already are being tested for their lignocellulosic activities in connection with our energy program. The Collection, dating from 1944 to 1976, is a valuable addition of many genera and species new to the NRRL Collection.

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HORTICULTURAL AND SPECIAL CROPS LABORATORY

L. H. Princen, Chief

Research Leaders: R. Kleiman, J. A. Rothfus, C. R. Smith,
and H. L. Tookey

A. BREEDING AND PRODUCTION - FORAGE CROPS FOR HAY, PASTURES, AND OTHER USES INCLUDING TURF

1. Chemicals in Tall Fescue Affecting Livestock Health and Forage Utilization (S. G. Yates)

- a. Specific Objective: Continue separation, identification, and quantitation of constituents in toxic fractions from tall fescue.

Progress: Identities or tentative structures have been assigned to 18 of 37 components detected by gas chromatography-mass spectrometry (GC-MS) in an anion-rich fraction that reduces hoof temperature when administered intraperitoneally to cattle (20100-002-A). Major constituents of this fraction include shikimic, quinic, and malic acids, which cause temporary coronary band reddening, but not hoof temperature reduction, when administered as a mixture of pure compounds. A subfraction that reduces hoof temperature also contains pyroglutamic and pipecolic acids but apparently neither of these is responsible for the fraction's biological activity. The active anion fractions contain several unidentified compounds that, like pipecolic acid, are secondary constituents. From preliminary evidence, some of these appear analogous to neurologically active hydroxy acids. GC-MS analyses, however, account for only ca. 25% of the material in each fraction. Alternative methods of analysis should provide additional candidate agents.

Publication:

BUSH, L. P., J. A. BOLING, and S. G. YATES. Animal Disorders. In Tall Fescue, eds., L. P. Bush and R. C. Buckner, American Society of Agronomy, Madison, WI, Chapter 13, pp. 247-292, 1979.

2. Bioassay of Chemical Constituents of Tall Fescue Forage (Cooperative Agreement - University of Missouri)

- a. Specific Objective: Assay chemically defined preparations containing major components identified in toxic anion fractions from tall fescue.

Progress: Evaluation of biologically active substances from toxic tall fescue proceeded with the intraperitoneal infusion of additional subfraction factors into cattle. Pure compounds previously shown to produce inflamed coronary bands but no sustained reduction in hoof temperature were administered in combination with two newly identified components of a subfraction that lowered hoof temperature without reddening coronary bands. The new mixtures produced temporary inflammation of the coronary band but did not lower hoof temperature. These assays complete tests of major components identified by GC-MS; they leave several secondary constituents and larger or more polar compounds to be identified and evaluated.

In winter, anion-rich fractions cause the greatest physiological disturbance in experimental cattle, but in summer, with animals at the upper limit of their ability to control body temperature, cation-rich fractions seem to produce more stress.

Statistical analyses of videothermometry data from the whole-animal experiments produced new knowledge of the significance of temperature-lowering factors in fescue foot. These analyses: (1) identified optimum times for collecting weighted coronary band temperature (WACBT) data, (2) demonstrated that WACBT values of affected animals are unaltered by ambient temperature, and (3) provided justification for assigning to ambient effects the WACBT value variations of nonpositive experimental animals. They also suggest that experimental animals should be matched in terms of their WACBT response to change in ambient temperature.

Publication:

YATES, S. G., J. A. ROTHFUS, G. B. GARNER, and C. N. CORNELL. Videothermometry for Assay of Fescue Foot in Cattle. Am. J. Vet. Res. 40(8) (1979):1192-1196.

Report:

YATES, S. G. Videothermometry for Assay of Fescue Foot in Cattle. Presented at the Rumen Function Conference, Chicago, Illinois, November 28-29, 1979.

B. INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION,
AND DOCUMENTATION OF PLANT GERMPLASM

1. Development of Jojoba as a Crop for Arid Lands (J. A. Rothfus)
 - a. Specific Objective: Characterize jojoba germplasm.

Progress: Germination studies evidenced substantial variation in the viability of jojoba seed collected from wild stands at different

locations and stored under different conditions. Whether variation is due in part to genetic variation remains uncertain. Seeds carried typical desert microflora at various levels. Germination rates were highest with seeds that were dried carefully and relatively free of microorganisms. Seeds stored fresh in air-tight containers performed poorly. Initial seedling growth was also affected by microorganisms and culture conditions. Correlations between seed size or density and oil content were developed to provide an additional basis for planting seed selection. Extracts of single leaves from male and female jojoba plants exhibited subtle spectrophotometric differences that remain uncharacterized in terms of utility in sexing immature plants.

- b. Specific Objective: Determine properties of jojoba-derived materials and aid in jojoba technology transfer.

Progress: Prospects for use of jojoba oil in soaps and shampoo formulations were defined by examining the oil's compatibility with a broad range of commercial soapstocks and detergents.

Publication:

MIWA, T. K., J. A. ROTHFUS, AND E. DIMITROFF. Extreme-Pressure Lubricant Tests on Jojoba and Sperm Whale Oils. J. Am. Oil Chem. Soc. 56(8) (1979):765-770.

Reports:

MIWA, T. K. Jojoba Oil as a Replacement for Sperm Whale Oil. Presented at the AOCS/JOCS meeting, San Francisco, California, April 29-May 3, 1979.

MIWA, T. K. Properties and Uses of Jojoba Oil. Presented at the Jojoba Conference, Phoenix, Arizona, July 20-21, 1979.

MIWA, T. K. Jojoba Oil. Presented at the American Oil Chemists' Society, North Central Section, meeting, Chicago, Illinois, March 28, 1979.

2. Chemical Analysis of Uncultivated Plants (R. Kleiman)

- a. Specific Objective: Screen seeds of incoming collections for oil and protein and for novel seed oil constituents.

Progress: Three hundred forty-five seed samples were added to the collection. These samples included the Papaver bracteatum collection from the SEA-AR Regional Plant Introduction Station at Pullman, Washington, seeds collected in India and Australia, and those collected locally in the Peoria area. Of the 54 samples chemically

screened, a high of 39% protein was found in Gompholobium latifolium and 49% oil in Trichilia connaroides. Many indicated unusual constituents by thin-layer and gas chromatography and by spectral analysis. Of the 112 samples analyzed for fatty acid composition, 39 were Compositae, many of which contained trans-3 acids. Other unusual fatty acids were observed in seed oils of Dyckia montevidensis (cis-vaccenic), Calendula tomentosa (57% conjugated triene), Asclepias mellodora (8% 16:1), Cynanchum auriculatum (12% 16:1), Entandrophragma angoleuse (17% 16:1), Ephedra intermedia (30% cis-5 acids), and Cuphea micropetala (87% C₁₀ saturate).

- b. Specific Objective: Characterize novel constituents in seed oils and other plant components.

Progress: Discovered in the seed oil of Knema elegans (Myristiaceae) was a new class of anacardic acids with a terminal phenyl group on a C₁₀ or C₁₂ saturated, or a C₁₂ monoenoic connecting chain. These compounds coexisted with the previously known type of anacardic acids. Galbacin and two related lignans were also identified. trans-2-Hexadecenoic acid, previously unrecognized as a plant constituent, was isolated from Aster scaber seed oil.

- c. Specific Objective: Analysis of potentially new crops in cooperation with plant breeders.

Progress: In the continuing program to develop rapeseed as a crop for the northwestern United States, 172 high erucic acid/low glucosinolate Brassica lines were analyzed in cooperation with Oregon State University. Consistent erucic contents of more than 55% were found along with low glucosinolate values. Low erucic acid lines were analyzed for the University of Idaho. Twenty samples of Jessenia oenocarpus were analyzed in conjunction with the Botanical Museum of Harvard University. Fatty acid and amino acid composition in addition to oil percentage was determined.

- d. Specific Objective: Install and make operational a new mass spectrometer/computer system.

Progress: The new mass spectrometer has been installed and is 90% operational.

- e. Specific Objective: Develop methods for analysis and characterization of plant materials.

Progress: Selectivity of HPLC columns toward triglyceride separations was determined. GC-MS methods to determine polysaccharide structure and to quantitate mycotoxins in grains were accomplished.

- f. Specific Objective: Complete data bank entering into laboratory computer of the NRRC seed collection and associated chemical information.

Progress: All essential data from the over 14,000 seed accessions have been entered onto computer disk storage. Included are inventory and source information and chemical data. Programs have been implemented to access the data.

- g. Specific Objective: Develop methods for processing Vernonia galamensis seed to produce a high-epoxy, low-free acid oil.

Progress: Lipase may be inactivated by heating at 200°F and 14% moisture; treatment of the seed with heat and moisture also facilitates fiber removal. In preliminary tests, the seed oil shows promise as a coatings material, forming baked films of good flexibility, hardness, adhesion, and resistance to chipping, acid, alkali, surfactant, and organic solvent.

Publications:

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DAXENBICHLER, M. E., G. F. SPENCER, AND W. P. SCHROEDER. 3-Hydroxypropylglucosinolate, A New Glucosinolate in Seeds of Erysimum hieracifolium (L.) and Malcolmia maritima (L.) R. Br. Phytochemistry, in press.

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PLATTNER, R. D., K. L. PAYNE-WAHL, L. W. TJARKS, AND R. KLEIMAN. Hydroxy Acids and Estolide Triglycerides of Heliophila amplexicaulis L.f. Seed Oil. Lipids 14 (1979): 576-579.

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SHOTWELL, O. L., G. A. BENNETT, M. L. GOULDEN, R. D. PLATTNER, AND C. W. HESSELTINE. Survey of Grain Sorghum for Zearalenone Aflatoxin and Ochratoxin. J. Assoc. Off. Anal. Chem., in press.

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SPENCER, G. F. AND M. E. DAXENBICHLER. Gas Chromatography-Mass Spectrometry of Nitriles, Isothiocyanates, and Oxazolidinethiones Derived from Cruciferous Glucosinolates. J. Sci. Food Agric., in press.

Reports:

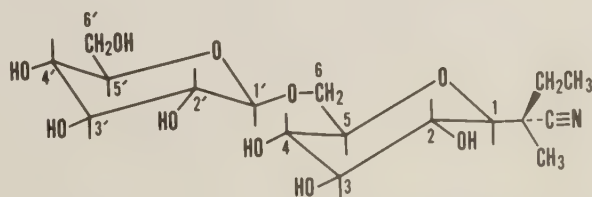
PAYNE-WAHL, KATHLEEN, R. KLEIMAN, and I. M. CULL. trans-3-Enoic Acids in Seed Oils of the Compositae. Presented at the Illinois State Academy of Science Meeting, Carbondale, Illinois, April 20-21, 1979.

PLATTNER, R. D. AND G. F. SPENCER. High Performance Liquid Chromatography of Triglycerides: Controlling Selectivity with Silica and Reverse Phase Columns. Presented at the AOCS meeting, San Francisco, California, April 29-May 3, 1979.

3. Biologically Active Plant Constituents for Pest Control and Medicine (C. R. Smith)

- a. Specific Objective: Detect, isolate, and characterize constituents of seeds and other plant parts that have potential value as pest control agents, or that have other biological activities beneficial to agriculture.

Progress: Large-scale fractionation work on Trewia nudiflora seed extract has culminated in the isolation of four new maytansinoid compounds which have potent antitumor properties as well as larvicidal activity against the European corn borer. (Maytansinoids, a group of ansa macrolide alkaloids, have been found previously in only two plant families and not in Euphorbiaceae.) Two of the new alkaloids are believed to incorporate a novel macrocyclic ring in addition to the one characteristic of maytansinoids. Further fractionation of the extract of Cephalotaxus mannii (old name) has provided three new antitumor compounds with the possibility of several additional ones; one of the new active compounds has been characterized as 19-hydroxybaccatin III. The larvicidal principles of Thevetia thevetioides seed have been isolated and identified as neriifolin and 2'-acetylneriifolin. Neriifolin, by far the more potent of the two, has been isolated in sufficient quantity for limited field trials with the European corn borer. Compounds responsible for defatted linseed meal's unique property of protecting livestock against selenium toxicity have been fully characterized. These compounds--linustatin and neolinustatin--are cyanogenic glycosides; neolinustatin has the following structure:



Extracts of diseased peach tree wood have been prepared which are found to be strongly attractive to the female peach tree borer and

also are oviposition initiators (bioassays by Dr. David Reed, SEAR, Vincennes, Indiana). The fraction of Lithospermum arvense most active as a mosquito larvicide contains a mixture of fatty acids and keto acids. Work on Berria ammonilla and Apium sellowianum has been discontinued as they appeared unpromising in our corn borer larval assay after further fractionation. Cooperative work with Dr. Alan Galsky, Bradley University, has (within the scope of compounds thus far tested) given promising results in the use of potato disks infected with crown gall tumors as a screen for anti-leukemic activity.

Publications:

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McLAUGHLIN, J. L. B. FREEDMAN, R. G. POWELL, AND C. R. SMITH, JR. Neriifolin and 2'-Acetylneriifolin: Insecticidal and Cytotoxic Agents of Thevetia thevetioides. J. Econ. Entomol., in press.

MILLER, R. W. A Brief Survey of Taxus Alkaloids and Other Taxane Derivatives. J. Nat. Prod., in press.

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SMITH, C. R., D. WEISLEDER, R. W. MILLER, I. S. PALMER, AND O. E. OLSON. Linustatin and Neolinustatin: Cyanogenic Glycosides of Linseed Meal that Protect Animals Against Selenium Toxicity. J. Org. Chem. 45 (1980):507-510.

4. Major Fatty Acids from Indian Seed Oils and Their Possible Industrial Use
(P.L. 480 Grant - Muslim University, Aligarh, India)

- a. Specific Objective: Screening Indian flora for unusual and potentially useful lipid constituents; synthesis of potentially useful derivatives.

Progress: The reaction of trans-13,14-epithiodocosanoic acid with BF_3 -etherate in DMSO yields a mixture of 13-mercapto-trans-14,15-docosenoic and 14-mercapto-trans-12,13-docosenoic acids. In contrast, the same starting material, upon treatment with BF_3 -methanol, gives 13(14)-mercapto-14(13)-methoxydocosanoate. Routes to fatty aziridines and oxathiolanes have been explored. Certain seed oils (Polyalthia longifolia, Cardiospermum sp., Heliotropium supinum, Semicarpus anacardium) have repellent action towards a grain weevil (Tribolium castaneum).

C. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY
TO IMPROVE CROP PRODUCTION

1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals
(N. E. Delfel)

- a. Specific Objective: Determine optimum harvest time for field grown Cephalotaxus trees.

Progress: Four of five trees sampled seven times during the year produced no antitumor alkaloid esters, even though two were known to have contained esters several years earlier. These trees are growing at the northern limit of their range and may have been adversely affected by recent harsh winters. In the fifth tree, which as grown in an unheated greenhouse, the isoharringtonine level in the stem increased sixfold from summer to early spring and then fell precipitously; the level in the leaves remained fairly constant. Homoharringtonine, on the contrary, was found only in the spring in either stems or leaves.

- b. Specific Objective: Reestablish new lines of C. harringtonia callus with normal antitumor alkaloid synthesis.

Progress: Young seedling C. harringtonia plants have been obtained and callus cultures initiated. These are not yet sufficient for chemical analysis.

- c. Specific Objective: Attempt to alter alkaloid biosynthesis by changes in culture conditions.

Progress: Alkaloid biogenesis in Cephalotaxus cells may require long days. Cultures that stopped such biosynthesis did so after transfer from 16 hours illumination in the laboratory to 12 hours

illumination in a growth chamber. Callus grown under continuous illumination still appears to produce the normal Cephalotaxus alkaloids; that grown under 12-hour light produced none detectable by GLC. Results await confirmation by alternate methods.

- d. Specific Objective: Identify unknown metabolites produced by aberrant C. harringtonia callus.

Progress: Six of the 14 major components have been identified by GC-MS: linoleic, oleic and palmitic acids, and campesterol, stigmasterol, and β -sitosterol. The three sterols, which are also present in tissues producing the normal alkaloids, showed an interesting correlation with the amounts of cephalotaxine and homoerythrina alkaloids.

- e. Specific Objective: Localize or quantitate alkaloids in cultures or whole plants via immunochemical techniques.

Progress: Callus cultures of Datura ferox and D. innoxia were initiated for these studies. The D. ferox cultures contained both atropine and scopolamine at levels 1/100 and 1/10 respectively, that of field-grown plants. The plants were also studied histochemically with three different chemical reagents for later comparison to immunochemical results. Atropine hemisuccinate (AHS) has been successfully attached to bovine serum albumin (BSA) to make an antigen suitable for anti-atropine antibody production. Rabbits have been injected with this AHS-BSA, and the antibodies so produced are being characterized by immunodiffusion and by crossed-rocket immunoelectrophoresis.

Publications:

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D. TECHNOLOGIES FOR FOOD AND FEED
USES OF FIELD CROPS

1. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

- a. Specific Objective: Investigate thermal behavior and polymorphism of dienoic and trienoic acids.

Progress: Mono- and dienoic fatty acids, C₁₈-C₂₂, and their methyl esters exhibit multiple endotherms and exotherms when examined by differential scanning calorimetry. Corresponding saturated acids, esters, and alkanes generally produce single sharp endotherms or exotherms upon heating or cooling. Related trienoic and tetraenoic compounds, in addition, tend to undergo cold-crystal reorientations seldom exhibited by their monoenoic counterparts. These reorientations, which apparently occur at specific rates and at preferred temperatures, evidence a certain, though still indefinite, correlation between structure and low-temperature behavior.

Initial studies on triglycerides of polyunsaturated acids, including geometrical and specially deuterated isomers, disclose α - and β -forms and unstable β' -forms, but have yet to evidence significant isotope effects on thermal properties as assessed by DSC.

Long-chain alcohols show two different melting forms that, unlike the multiple melting forms exhibited by acids and triglycerides, cannot be altered by variations in heating or cooling rates.

- b. Specific Objective: Obtain Raman spectra for polymorphic forms of triglycerides with known structures and previously characterized thermal properties.

Progress: Raman spectra of single-acid (C₁₇ or C₁₈) saturated triglycerides exhibit characteristic changes in band intensities consistent with increased crystal order as these materials are heated through α -, β' -, and β -polymorphic conformations. Intensity changes in bands that reflect skeletal optical modes of the odd-chain triglyceride suggest, however, that its β' -form may involve relatively less trans orientation than is present in either the α - or β -polymorph. Typical band broadening and shifts to lower frequency occur upon heating through the melt transition.

- c. Specific Objective: Continue computer modeling of triglyceride structures.

Progress: Hexagonal spacings were determined for one of four possible α -form conformations of triarachidin by analyzing molecular interactions in a computergenerated geometric model that allows

nonsynchronous oscillation of hydrocarbon chains about their long axes. Computed interaction maxima indicate that the preferred arrangement for this particular α -form conformation consists of molecules oriented through x and z crystal axes as mirror images of the central triglyceride molecule. Initial results with a second triarachidin conformation suggest, however, that more than one α -form conformation can be accommodated in the hexagonal arrangement.

Publications:

EISSLER, R. L. AND J. W. HAGEMANN. Thermodynamic Properties of Fatty Acids. In "Fatty Acids," edited by E. H. Pryde, AOCS Monograph, Chapter 9, pp. 173-194. 1979.

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Report:

CHANG, S. P. Thermal Behavior of Ordered and Disordered Crystals from Fatty Acids, Esters, and Alkanes. Presented at the ISF/AOCS World Congress, New York, April 27-May 1, 1980.

2. Analysis for Improved Soybean Quality (R. Kleiman)

- a. Specific Objective: Determine oil and protein content of soybean samples in order to develop improved varieties.

Progress: About 13,000 samples were received from public soybean breeders throughout the United States and Canada. These samples were examined for their oil and protein content by the infrared reflectance method. Samples (4,150) of soybean plant parts were analyzed for nitrogen using a rapid digestion-colorimetric autoanalyzer system. These samples included roots, stems, leaves, pods, and seed and exhibited protein levels from 5 to 50%.

- b. Specific Objective: Provide fatty acid composition of selected soybean samples in order to lower the linolenic acid content through plant breeding.

Progress: Fatty acid composition was determined on 1,450 soybean samples. Included in these samples were those from the "Wild"

collection from SEA-AR, University of Illinois and Plant Introduction from SEA-AR, Stoneville, Mississippi. Linolenic acid content as high as 25% was found in the "Wild" samples.

- c. Specific Objective: Develop rapid analytical procedures for soy protein in order to raise the methionine and cysteine content through breeding.

Progress: The GC-cyanogen bromide procedure for determination of methionine was evaluated and was found to give consistently low values. At this point this procedure is not feasible for rapid determination of this amino acid.

- d. Specific Objective: Develop and improve chemical analytical methods for soybeans.

Progress: Gas chromatographic conditions were developed to allow analysis of up to 300 samples per day for fatty acid composition. One quarter of the usual sample size is being used for digestion in nitrogen determination. A second infrared reflectance analyzer was interfaced to the laboratory computer.

Reports:

The Uniform Soybean Tests, Northern States 1978. USDA, ARS, and State Agricultural Experiments Stations, West Lafayette, IN 47907.

The Uniform Soybean Tests, Southern States 1978. USDA, ARS, and State Agricultural Experiment Stations, Stoneville, MS 38776.

E. RECLAMATION AND REVEGETATION OF LAND AREAS DISTURBED BY MAN

1. Trace Element Uptake and Distribution in Agricultural Crops Grown on Disturbed Lands (K. D. Carlson)

- a. Specific Objective: Evaluate effect of sewage sludge application to stripmined land on the growth of crambe and kenaf.

Progress: Crambe and kenaf were grown on stripmine soil in three replications of four treatments: Control (C, no soil amendment), commercial fertilizer (CF), 50 as is ton/acre of dewatered sludge (S₅₀), and 100 as is ton/acre sludge (S₁₀₀). Statistically significant differences in plant densities (increasing order: S₁₀₀<S₅₀<C<CF) were observed between all treatments for crambe, but for kenaf only between sludge (S₁₀₀, S₅₀) and non-sludge treated (C, CF) plots.

Kenaf yields on S₁₀₀ plots were significantly lower than on the other plots. The experiment will be repeated in 1980.

- b. Specific Objective: Determine uptake by these industrial crops of heavy metals that may be contained in sewage sludge.

Progress: Soil, sludge, and plant samples (seed, stem, and root) were collected from the 1979 plantings and are being prepared for heavy metal analyses.

F. UTILIZE, MANAGE, AND CONSERVE SOIL FERTILITY FOR
INCREASED PRODUCTION AND NUTRITIONAL QUALITY OF
PLANTS AND ANIMALS

1. Improve and Implement the Determination of Isotopic Nitrogen in Soil Samples (R. Kleiman)

- a. Specific Objective: Install and make operational isotope ratio mass spectrometer.

Progress: A Varian MAT 250 was delivered in August 1979. The equipment was rapidly made operational and personnel became expert in its operation. An all-glass nitrogen generation and sample introduction system was designed and fabricated.

- b. Specific Objective: Start actual analysis of samples that will be submitted by other SEA locations and cooperating scientists for their N-fixation and N-cycle research programs.

Progress: Standards were obtained and tested in the system with good results. Samples started to arrive from cooperators.

G. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Cattle Feeding Tests of Crambe Meal as a Protein Concentrate (L. H. Princen)

- a. Specific Objective: Obtain FDA clearance for using Crambe meal in beef cattle rations.

Progress: Word has been received that FDA has accepted our feeding trials and analytical data for use of Crambe meal as part of the protein diet for beef cattle rations, and that FDA's legal department is preparing the announcement for publication in the Federal Register. No additional experiments are required or planned.

Publication:

PERRY, T. W., W. F. KWOLEK, H. L. TOOKEY, L. H. PRINCEN, W. M. BEESON, AND M. T. MOHLER. Crambe Meal as a Source of Supplemental Protein for Growing-Finishing Beef Cattle. J. Anim. Sci. 48 (1979): 758-763.

2. Natural Toxicants in Horticultural Crops and Cruciferous Foods (H. L. Tookey)

- a. Specific Objective: Determine biological activity of glucosinolate aglucon products that occur in significant levels in cruciferous vegetables.

Progress: Nine aglucons were found to be nonteratogenic in the rat. However, three of these decreased fetal size, and one induced rolling seizures in the pregnant rat.

- b. Specific Objective: Discover convenient sources of glucosinolate products needed for biological testing.

Progress: 1-Cyano-2,3-epithiopropene may be prepared conveniently from seeds of Brassica juncea. Several other species identified as rich sources of specific glucosinolates are being grown to increase seed stocks.

- c. Specific Objective: Determine effects of edaphic conditions on the glucosinolate content of cabbages.

Progress: In cooperation with the University of Wisconsin, cabbages have been grown under various stresses of moisture and fertilizer. The first-year crop has been harvested and analyses are in progress.

- d. Specific Objective: Provide data base for evaluation of newly developed cultivars in regard to their content of glucosinolates.

Progress: Glucosinolates in 23 named cultivars of radish range from 411 to 2,184 ppm, fresh weight. A survey of crucifer vegetables other than cabbage is continuing.

- e. Specific Objective: Develop and validate a method for the assay of falcarinol, a toxicant found in carrot roots.

Progress: An improved analytical method recovers 70% of falcarinol added to carrot roots.

Publications:

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VanETTEN, C. H., H. L. TOOKEY, and M. E. DAXENBICHLER. Glucosinolates. In Toxic Constituents of Plant Foodstuffs, 2nd Edition, ed., I. E. Liener, Academic Press, 1980, pp. 103-142.

OILSEED CROPS LABORATORY

H. J. Dutton, Chief

Research Leaders: E. A. Emken, E. N. Frankel, T. L. Mounts,
E. H. Pryde, and W. J. Wolf

A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Increased Photosynthetic Efficiency of Plants Through Yellow Chloroplast Pigments (H. J. Dutton)

- a. Specific Objective: Study color and energy transfer changes in Phaeodactylum tricornutum and Chlorella pyrenoidosa with temperature.

Progress: Heated to 60 C the brown diatom Phaeodactylum tricornutum undergoes an instantaneous change in absorption spectrum and change in color from brown to green. Even more dramatic is the change in fluorescence excitation spectrum where total loss of energy transfer from fucoxanthin to chlorophyll a occurs and the excitation spectrum is quite identical with that of the extracted pigments where no energy transfer also occurs. Heating appears to destroy the spatial arrangement in the chloroplast of exterior fucoxanthin and interior chlorophyll a by randomly dissolving them in cell membrane lipids and thereby obviating energy transfer capability. Heating Chlorella pyrenoidosa (model for the green plant) to 60 C causes little change in absorption spectrum or visible color; however, the energy transfer from chlorophyll b to chlorophyll a was strikingly diminished.

- b. Specific Objective: Apply the mass spectrometric system for determining isotopes of oxygen and carbon dioxide to the study of photorespiration in algae.

Progress: The Bendix Time-of-Flight mass spectrometer is now able to monitor isotopes of oxygen ($^{18}\text{O}_2$ and $^{16}\text{O}_2$) and carbon dioxide ($^{13}\text{CO}_2$ and $^{12}\text{CO}_2$) accurately and with high sensitivity. Measurements of photorespiration have been made for Chlorella pyrenoidosa and Scenedesmus obliquus. These organisms show no Warburg effect at air saturated levels of CO_2 . However, rates of CO_2 evolution in the light have been shown to be twice the dark rate. Carbon dioxide "gulps" and post illumination "bursts," generally thought to be an indication of photorespiration, have been shown to be artifact of $\text{CO}_2/\text{HCO}_3^-$ equilibrium. Carbonic anhydrase has been used to mediate this rate determining step in order to accurately observe photorespiration.

- c. Specific Objective: Isolate light harvesting carotenoid-chlorophyll c-chlorophyll a protein complex without disrupting its energy transfer capacity.

Progress: A highly fluorescent light harvesting carotenoid-chlorophyll c-chlorophyll a-protein moiety was separated from the marine diatom Phaeodactylum tricornutum which exhibited efficient energy transfer from carotenoids (excited in the region of 470 nm) to chlorophyll a (emission at 680 nm). The separation process involved cell homogenization by an abrasion method, subsequent centrifugations and minimal treatment of disrupted chloroplasts with sodium lauryl sarcosinate (3 molecules per 10,000 daltons of protein at 5 C, 1 hr in the dark). Criteria for energy transfer was based on similarities of absorption and quantum-corrected excitation spectra at low complex concentrations (0.035 mg chl a/l). Molar amounts of pigments in the light harvesting complex (determined by HPLC) based on carotene as 1 are: chlorophyll c, 3; chlorophyll a, 3; fucoxanthin, 12; neofucoxanthin, 4. Only minor amounts of diadinoxanthin, and diatoxanthin were found. Differences in ratios of chlorophylls a/c in the initial cells and in the isolated fraction (2.5:1 as compared to 1:1) was regarded as evidence that a fractionation had occurred during the separation process. Separation of reaction centers from the complex by action of detergent could explain the increase in the chlorophyll a to c ratio. Absence of reaction centers in the complex was shown by low temperature (77 K) emission studies. Dependence of energy transfer on structural arrangements of chlorophyll a and carotenoids was demonstrated. Disruption of pigment-protein molecular organization by heating in 1% sodium dodecylsulfate altered the excitation spectrum of the complex to a form which resembled that of chlorophyll a.

- d. Specific Objective: Continue the characterization of a polarographic signal associated with superoxide generation in chloroplasts.

Progress: Earlier results had indicated that chloroplasts that were illuminated with flashes gave rise to signals observable with a positive biased rate electrode. These signals originated from photosystem I and were believed due to superoxide (O_2^-). Studies, using the rate electrode for polarographic measurements, indicate that O_2^- itself is not being detected. Instead, O_2^- acts as an intermediate in the reduction of an endogenous charge carrier with a 340 mV redox potential, and this carrier is detected. Both methyl viologen and ferricyanide at low concentrations were found to enhance this signal. Cycle-of-two oscillations in this signal and amplitude changes as a function of electrode potential during dark adaptation solidify the relationship of this signal to electron flow between photosystems I and II.

- e. Specific Objective: Compare chloroplast pigment contents of chlorophyll deficient genotypes at various stages of growth to demonstrate the expression of individual genes (Coop SEA-AR, U of Illinois).

Progress: Using improved techniques of sample preparation and analysis, the chloroplast pigment content of 11 different chlorophyll deficient genotypes (homo- and heterozygates) were analyzed at various stages of growth. Comparisons between normal and mutant genotypes were made to demonstrate the expression of pigment deficiency brought about by individual genes. The additional pigment analysis further confirmed and expanded the genetic model of greening and strongly suggested a correspondence between reduced violaxanthin content and increased lutein in the mature plant.

Publications:

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ESKINS, K. AND H. J. DUTTON. Sample Preparation For High-performance Liquid Chromatography of Higher Plant Pigments. Anal. Chem. 51 (1979):1885-1886.

Report:

JURINIC, P. Effects of Excitation Intensity on the Flash Yield Pattern of Photosynthetic Oxygen Evolution. Seventh Annual Meeting, American Society for Photobiology, Asilomar Conference Grounds, Pacific Grove, California, June 11-16, 1979.

2. Photosynthetic Pigments and Primary Photoacts for Increasing Efficiency and Yield of Crops (H. J. Dutton)

- a. Specific Objective: Correlate the spectral absorption of chloroplast pigments with the action spectra of carotenoid sensitized photosynthesis in green and yellow soybean isolines.

Progress: Reflectance spectra of green and yellow soybean mutants have been measured with a spectrophotometer equipped with an integrating sphere and show differences corresponding to visual colors. Peaks corresponding to chlorophyll a, chlorophyll b and carotenoids have been identified so that fluorescence excitation action spectra can now be measured and interpreted.

- b. Specific Objective: Investigate photorespiratory losses in green and yellow soybean isolines (Coop SEA-AR, University of Illinois) through stable isotopes of oxygen and of carbon in carbon dioxide.

Progress: Whole cells have been isolated from soybean leaves in preliminary experiments. Oxygen and carbon dioxide exchanges have been measured on suspensions of these cells using stable isotopes $^{13}\text{CO}_2$, $^{12}\text{CO}_2$, $^{18}\text{O}_2$ and $^{16}\text{O}_2$ on the Bendix mass spectrometer with its 100-fold improved accuracy and sensitivity.

- c. Specific Objective: Investigate dependence of energy transfer on structural arrangements of chlorophylls and carotenoids in model systems and in chlorophyll-caroteno-proteins isolated from chloroplasts.

Progress: Chlorophyll a and chlorophyll b, alone and in combination were adsorbed on the hydrophobic surfaces of pellicular, C-18, silica-glass microbeads. Relative concentrations of pigments adsorbed onto the substrate from methanol solution were controlled by varying the proportion of water in the solvent. Fluorescence excitation spectra showed a relatively high energy transfer from chlorophyll b to chlorophyll a when both were adsorbed in equal amounts on the same surface so that the average area occupied by a molecule was 5.6 nm^2 . Less energy transfer occurred at greater intermolecular spacings.

- d. Specific Objective: Monitor primary light reactions of the oxygen evolving reaction center and the effects of magnesium ion on the arrangement of this reaction center's antenna pigments.

Progress: A method was developed for indicating inhomogeneity in antenna pigment aggregation based on changes in the flash-yield pattern of oxygen evolution at various flash intensities. This technique was applied to chloroplast samples prepared with and without divalent salts. It was found that samples with divalent salts were similar to controls and had inhomogeneous aggregation of pigments. Samples lacking divalent salts had homogenous pigment aggregates of small size. The effects of divalent salts in chloroplasts are supposed to mimic photosystem I and II preillumination light effects as observed in whole plant cells such as algae. However, no significant changes in inhomogeneity of pigment aggregation in algae occurred due to preillumination light. It was concluded

that the use of salt effects in chloroplasts as a model for preillumination effects in whole plant cells may lead to erroneous conclusions.

- e. Specific Objective: Compare changes in contents of chlorophyll and carotenoid pigments during greening and maturation of various yellow and green isolines of soybeans.

Progress: A new method of analysis for chlorophyll precursors in etiolated systems was devised and refined. New "chlorophyll a-like" compounds were observed as intermediates in the greening process.

- f. Specific Objective: Follow changes in partitioning of photosynthate in soybeans between vegetative, reproductive, and nitrogen fixing functions (NC-141 cooperation).

Progress: Availability and proximity of sources of $^{11}\text{CO}_2$ (20 minute half life) and feasibility of use of certain commercial equipment are being explored for visualizing the partitioning of the photosynthate using the position of ^{11}C .

Report:

JURSINIC, P. Inhomogeneity of Photosynthetic Units as Indicated by the Oxygen Flash Yield Pattern. BARC Lecture Series, Beltsville, Maryland, October 5, 1979.

B. TECHNOLOGIES FOR FOOD AND FEED
USES OF FIELD CROPS

- 1. Effects of Isoelectric Precipitation and of Heat on Soybean Proteins
(W. J. Wolf)

- a. Specific Objective: Complete characterization of mono-, di-, and triglycerides separated from soy protein isolate and determine their effects on foaming of soy protein isolates.

Progress: Fatty acid analyses of the isolated glycerides by gas chromatography revealed slow eluting compounds suspected to be unsaturated fatty acids modified by oxidation. Oxidation of the neutral lipid fractions was confirmed by absorbance measurements at 234 nm. Oxidation was noted primarily in the monoglycerides with less in the diglycerides and one in the triglycerides. Foaming tests with alcohol-extracted isolated proteins indicated that the mono-, di-, and triglycerides found in the neutral lipid fraction are largely responsible for the poor foam stability of isolates prior to their extraction with aqueous alcohol.

- b. Specific Objective: Improve conditions for separation of the acid-sensitive fraction (ASF) of soybean proteins and for dissociation of noncovalently bound nonprotein constituents associated with ASF.

Progress: Gel filtration of the water extractable soybean proteins on acrylamide-agarose beads resulted in elution of most of the ASF at the column void volume and separation from the nonacid-sensitive proteins. Rechromatography of the isolated ASF on more porous acrylamide-agarose gels resulted in separation into several fractions. The ASF appears to preferentially bind compounds causing undesirable flavors and colors in soy proteins. These compounds are partially dissociated from the proteins in ASF by treatment with sodium dodecyl sulfate followed by gel filtration on Sephadex G-200.

- c. Specific Objective: Refine liquid chromatography method for analysis of phenylthiohydantoin derivatives of amino acids.

Progress: A method for separating phenylthiohydantoin (PTH) derivatives by liquid chromatography was modified so that 21-23 PTH amino acids can now be separated with an isocratic solvent system in less than 30 minutes. This technique is superior to gas chromatography since the compounds are stable and all of the common PTH's can be analyzed without further derivatization as is necessary for the nonvolatile PTH amino acids.

- d. Specific Objective: Isolate and identify the compound(s) responsible for fluorescence of soy protein products.

Progress: Extraction of soy flours, concentrates, and isolates with solvents such as aqueous alcohols, yielded extracts with an absorption peak at 262 nm. These extracts also fluoresced at 440 nm when excited at 360 nm. Absorbance at 262 nm and fluorescence at 440 nm correlated with each other but their intensities varied with type of soy product. The compound(s) responsible for absorbance and fluorescence are believed to be isoflavones and differences in content between products are probably the result of natural variations in soybeans plus processing differences. These results provide a tentative explanation for variability in fluorescence of various soy protein products as noted in our previous description of a fluorescent method for estimating the amount of soy protein in meat-soy blends.

- e. Specific Objective: Evaluate newly developed column(s) for high performance liquid chromatography (HPLC) of soybean proteins.

Progress: A new column designed specifically for HPLC of proteins became available recently and has been evaluated for separation of soy proteins. Preliminary results indicate that separations occur primarily according to molecular size, but other factors such as adsorption may also influence results. With further development, the technique may be a useful and rapid analytical tool for monitoring protein fractionations.

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NASH, A. M. AND W. J. WOLF. Effect of Aging Soybean Globulins on Their Solubility in Buffer at pH 7.6. Cereal Chem. Submitted for publication.

WOLF, W. J. Soybean Proteins in Human Nutrition. World Soybean Research Conference II. Submitted for publication.

Report:

WOLF, W. J. Protein Fractionation in Soybeans and Its Importance for Tofu Making. Presented at 2nd Soycrafters of North America Conference, Amherst, Massachusetts, July 1979.

2. cis-Bond Forming Hydrogenation and Autoxidation Studies to Improve Edible Soybean Oil (E. N. Frankel)

- a. Specific Objective: Study the mechanism of flavor deterioration in vegetable oils caused by oxidation.

Progress: Secondary products of autoxidized methyl linolenate were separated by high-pressure liquid chromatography to clarify the structures of these potential flavor and odor precursors. Autoxidation at 80 C produced significant amounts of allylic epoxy esters, and autoxidation at room temperature gave evidence of cyclic peroxides

(also known as endoperoxides). These cyclic compounds are related to the prostaglandins which are potent biologically active lipid oxidation products. Model allylic epoxides, diepoxides and endoperoxides have been synthesized to aid in the identification of the secondary oxidation products of methyl linolenate.

- b. Specific Objective: Evaluate immobilized cis-producing catalysts for the hydrogenation of soybean oil.

Progress: Soluble siloxane polymer-chromium carbonyl complexes were previously used to catalyze the stereoselective hydrogenation of methyl sorbate to cis-3-hexenoate. Although these new polymeric catalysts were thermally stable and could be recycled two or three times they were not effective for the hydrogenation of soybean oil. Now the polyphenyl siloxane was chemically bonded to silica to prepare insoluble chromium carbonyl catalysts that could be used at temperatures up to 210 C for the hydrogenation of soybean oil. The hydrogenated product contained 3.0 to 3.6% linolenate and 3 to 5% trans unsaturation. However, no recycling of these catalysts was possible. Similarly, the crosslinked polystyrene-chromium carbonyl complex catalysts have been improved by affording more recycling (six times in acetone and ten times in cyclohexane) with methyl sorbate but were ineffective with soybean oil. Metal analyses showed that chromium is solubilized in the hydrogenated products. Therefore, these types of complexes apparently behave like homogeneous catalysts.

Publications:

BUDOWSKI, P.,* I. BARTOV,* Y. DROR,* AND E. N. FRANKEL. Lipid Oxidation Products and Chick Nutritional Encephalopathy (*The Hebrew University, Rehovot, Israel). Lipids 14 (1979):768-772.

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FRANKEL, E. N., R. A. AWL, AND J. P. FRIEDRICH. cis Unsaturated Fatty Acid Products by Hydrogenation with Chromium Hexacarbonyl. J. Am. Oil Chem. Soc. 56 (1979):965-969.

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Reports:

FRANKEL, E. N. Analysis of Autoxidized and Photosensitized Oxidized Fats by Gas Chromatography-Mass Spectrometry. Presented at Joint Meeting of the American Oil Chemists' Society and the Japan Oil Chemists' Society, San Francisco, California, April 29-May 3, 1979.

FRANKEL, E. N. Lipid Oxidation. Presented at the Basic Symposium on Food Lipids. Institute of Food Technologist's Meeting, St. Louis, Missouri, June 8-9, 1979.

3. Mass Spectrometry of Deuterium Labeled Blood Lipids, Soybean Oil Odors, and Other Research Samples (W. K. Rohwedder)

- a. Specific Objective: Establish accuracy and sensitivity of mass spectroscopic methodology for analysis of mixtures containing three different deuterated fatty acid isomers.

Progress: A triple-label selective-ion-monitoring mass spectroscopic technique was developed which allows the concentration of each of three different deuterium labeled fatty acids isomers to be accurately determined in the presence of nonlabeled fats which occur in human blood lipids. Several combinations of deuterium labeled standards mixed with varying amounts of nonlabeled methyl oleate were used to test the accuracy and sensitivity of the technique and to compare the results to previously developed dual-labeled procedures. The standard deviation of the triple-labeled methodology was similar to that reported earlier for dual-labeled methodology. A comparison of standard deviation associated with molecular ion (M) and molecular ion fragments (M-31, M-32) confirmed previous observations that higher precision is obtained using the molecular ion fragments. Programming for a PDP-12 computer system was completed and tested with the Finnigan mass spectrometer. The system is functional and will permit mass spectroscopy data generated by selective-ion-monitoring to be collected and processed.

- b. Specific Objective: Complete identification of volatiles from heated trilinolein and begin process of identification of volatiles from tri-(cis-9, cis-15-octadecadienoyl)glycerol and trilinolenin which are formed during heating.

Progress: The mass spectra of volatiles from heated trilinolenin from three different sources are being analyzed in order to identify the compounds associated with the room odor produced by this compound. Similarly the volatiles from heated tri-(cis-9,cis-15-octadecadienoyl)glycerol (9-cis,15-cis-18:2) are being identified by mass spectral analysis. Many compounds previously found in heated hydrogenated soybean oil have been identified in trilinolenin and 9-cis,15-cis-18:2). Present work is concentrating on MS

spectra which contain data from more than one compound and on MS spectra of compounds for which there is no reference spectra available. Dinitrophenylhydrazine derivatives of 12 different aldehydes were obtained and the aldehydes regenerated for mass spectral analysis. Data on these aldehydes should provide reference spectra which can be used to identify some of the unknown and unusual aldehydes generated by heating trilinolenin and 9-cis,15-cis-18:2 triglycerides.

Identification of volatile compounds produced by heating--trilinolein and mixtures of trilinolein-tristearin, trilinolein-triolein, and trilinolein-triolein-tristearin in air was completed. Volatiles were collected, separated, and identified by gas chromatography-mass spectrometry. Major volatiles observed from each heated sample produced compounds unique to the autoxidation-decomposition of the trilinolein component and included: pentane, acrolein, pentanal, 1-pentanol, hexanal, 2- and/or 3-hexenal, 2-heptenal, 2-octenal, 2,4-decadienal, and 4,5-epoxydec-2-enal. When samples containing both trilinolein and triolein were heated, volatiles were produced that could be ascribed to each triglyceride. However, heated mixtures containing tristearin produced no observable volatiles that could be related to the oxidized saturated triglyceride. Minor volatiles identified from the heated trilinolein and its mixtures included: aliphatic acids, saturated and unsaturated aldehydes, primary and secondary alcohols, gamma lactones, furans, hydrocarbons, and methyl ketones.

- c. Specific Objective: Improve mass spectroscopy instrumentation and cooperate with other NRRC scientists for identification of natural and synthetic compounds by mass spectroscopy.

Progress: Modification of a Bendix gas chromatograph (GC) and the design and construction of the hardware necessary to interface the Bendix GC with the Nuclide mass spectrometer has been completed. The Bendix GC will replace the existing Packard GC and will improve the high resolution capabilities of the Nuclide mass spectrometer. As part of a continuing program to modernize and improve the mass spectroscopy facility and capabilities, replacement of vacuum tube electronics with solid state electronics has been initiated.

Cooperated with 17 NRRC scientists from various laboratories to determine the structure or confirm the identification of compounds by MS analysis. Samples analyzed included a wide range of natural and synthetic compounds isolated or synthesized in the course of research on a variety of projects. Many samples involved analyses of complexed mixtures which could not have been identified by any other instrumental technique.

Publications:

ROHWEDDER, WILLIAM K., WILLIAM F. KWOLEK, DARHAL J. WOLF, AND WAYNE L. EVERHART. Mass Spectrometric Analysis of Deuterium Dual Labeled Blood Lipids. Biomedical Mass Spectrometry 6 (1979):67-71.

SELKE, E., W. K. ROHWEDDER, AND H. J. DUTTON. Volatile Components from Trilinolein Heated in Air. J. Am. Oil Chem. Soc. Accepted 1979.

Report:

ROHWEDDER, W. K. Mass Spectrometric Analysis of Deuterium Triple-Labeled Blood Lipids. Presented at American Soc. for Mass Spec., Seattle, Wash., June 3-8, 1979.

4. Dietary Fibers and Residual Lipids in Defatted Soybean Protein Products (J. J. Rackis)

a. Specific Objective: Purify and characterize soybean peroxidase.

Progress: Crude soy peroxidase extracted from ground whole soybeans by sonication was purified 200-fold by consecutive ammonium sulfate precipitation, column chromatography on Bio-Gel P-4 and on DEAE-Sephadex and affinity chromatography on Sepharose CL-4B. Small amounts of impurities remain. The true peroxidative nature of the enzyme was established with quaiacol as the H-donor. Oxidative reactivity was equal to that of highly active horseradish peroxidase. Soy peroxidase shows a pH optimum at 5.5 and exhibits appreciable heat stability; 97% inactivation occurs at 70 C for 90 min or 80 C for 15 min. Three isoenzymes having identical molecular size but different charges were isolated as evidenced by disc gel electrophoresis. These isoenzymes apparently are glycoproteins.

b. Specific Objective: Identify oxidation products of soy phosphatidylcholine (PC) molecular species.

Progress: Soy PC molecular species were separated and purified by high pressure liquid chromatography (HPLC) and then oxidized in an aqueous emulsion at 37 C. The oxidation products were isolated and identified. In the model system, hydroperoxides (-OOH) were the only products found in the intact molecule. For example, oxidation of the two fatty acids in 18:2-18:2 PC, the major molecular species of soy PC, produced two hydroperoxides, 18:2OOH-18:2 PC and 18:2OOH-18:2OOH PC; and in all four geometric and positional isomeric forms. Preliminary kinetic data indicate that the overall rate of oxidation of the soy PC's is a function of the most labile species present.

- c. Specific Objective: Determine nondigestible content of soy products.

Progress: Successive pepsin and pancreatin digestion was used to determine the insoluble and soluble nondigestible content of the residue fraction, a byproduct resulting from the manufacture of soy protein isolates. The residue, representing 25 to 35% of the solids content of dehulled, defatted soy flakes contains 55.3% indigestible matter (21.9% insoluble and 33.4% soluble nondigestibles). Finding large amounts of undigested protein in the residue byproduct and previously analyzed hulls, soy flour, and soy protein concentrates was unexpected and may have nutritional significance.

- d. Specific Objective: Detect and isolate cyanogens in soybeans.

Progress: Work was initiated to evaluate two colorimetric methods for the detection of CN^- . A large number of modifications were explored in an attempt to remove substances that interfere with the quantitative analyses.

- e. Specific Objective: Develop protocol for studies on effects of trypsin inhibitor (TI) on rat pancreas.

Progress: A Cooperative Agreement, "Biochemical effects of vegetable and animal trypsin inhibitors in long-term animal feeding tests" was developed between NRRC, SEA-AR's Western Regional Research Center, Albany, CA, and University of Minnesota Agricultural Experiment Station (cooperator). Effect and mechanism of action of TI's on enzymes in the pancreas and histochemical changes in the pancreas and other organs will be evaluated in high protein and high fat diets. Rats will be sacrificed periodically for assay of metabolic changes in the pancreas and correlated with histochemical changes in a series of lifetime (2-year) feeding trials. A series of 1500 to 4000 pound batches of custom-processed soy protein products (3-soy flours; 3-isolates) and casein were prepared and analyzed for nitrogen, moisture, ash, amino acids, and TI activity. TI activity ranged from 3.5 to 33 mg/g sample. Microbial analyses revealed no mycotoxin-producing organisms in the Salmonella-free protein ingredients. All products were put into cold storage (2-4 C; 70% relative humidity) for the duration of the 2-year test. The nitrite (8.6-21.3 ppm) and nitrosamine (0.6-1.5 ppb) contents of the protein ingredients were not considered to be nutritionally significant. Interest in these substances arises from the fact that high levels of raw soy flour potentiate the pancreatic carcinogenic activity of nitrosamines in rats.

Publications:

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SESSA, D. J. Biochemical Aspects of Lipid-Derived Flavors in Legumes. J. Agric. Food Chem. 27 (1979):234-239.

Reports:

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RACKIS, J. J. Protease Inhibitors: Physiological Properties and Their Relationship to Food Quality of Soy Protein Products. Presented at Symposium on Antinutrients and Naturally Occurring Toxicants, American Chemical Society, Honolulu, Hawaii, March 31-April 5, 1979.

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5. Interactions Between Proteins and Oxidized Lipids of Soybeans (L. C. Wang)

- a. Specific Objective: Evaluate fluorescence techniques for measuring interactions between soybean proteins and compounds known to be lipid oxidation products.

Progress: Allylic enones and dienones known to be formed by autoxidation of methyl oleate and linoleate, respectively, were added to solutions of soybean protein isolates buffered at neutral

and alkaline pH's. The ketone-protein systems produced fluorescence at 445 nm when excited at 350 nm. However, the presence of interfering materials in soybean proteins (probably isoflavones) caused difficulties in the fluorescence assay. When the enones and dienones were mixed with purified soybean protein fractions (2S, 7S, 11S, and polymerized 7S) and excited at a shorter wavelength (280 nm), the mixtures fluoresced at 350 nm. The chromophore emitting at 445 nm is likely to be a Schiff base-imine system which is indicative of reaction between the ketones and soy protein, but origin of the emission observed at 350 nm is unknown.

- b. Specific Objective: Complete study of effects of ultrasonic treatment on physical properties of soybean proteins.

Progress: Water extracts of soybean meal prepared by ultrasonic treatment were examined by gel filtration, disc gel electrophoresis and ultracentrifugation. Gel filtration indicated that the proteins were aggregated by ultrasonic treatment. The ultracentrifuge revealed that aggregation involves conversion of 7S proteins into a 40-50S agglomerate which can be reversed by standing or dialysis against water. The agglomerate, however, appears stable in pH 7.6, 0.5 ionic strength buffer. This sensitivity of the 7S protein suggests some unique structural differences between it and the 11S protein, the other major storage protein of soybeans.

Publication:

WANG, L. C. Soybean Protein Agglomeration: Promotion by Ultrasonic Treatment. J. Agric. Food Chem. Submitted for publication.

6. Improving Food Quality of Soy Oil Products and Their Stability to Heat- and Light-Catalyzed Oxidation (E. N. Frankel)

- a. Specific Objective: Develop methodology and isolation techniques to monitor changes in heated oils caused by thermal oxidation.

Progress: In a collaborative study with Professor E. G. Perkins, University of Illinois, an improved gas chromatographic method was developed to quantitate cyclic monomers formed by the thermal oxidation induced during the deep-fat frying process in order to assess the potential toxicity of oils abused in this manner. Soybean oil and hydrogenated soybean oil were used to fry potato slices at 195 C continuously or intermittently for 52 and 104 hr periods. At chromatographic conditions sensitive enough to detect a cyclic monomer standard at concentrations lower than 0.5% by weight, no cyclic monomers were detected in any of the heated oil samples. However, when the samples were concentrated by removing a major portion of the straight-chain fatty acids by low-temperature crystallization, levels between 0.2 and 0.4% of materials with same

retention as cyclic monomers were detected in heated soybean oil, together with other unidentified materials. Previous studies of Professor Perkins with laboratory animals indicated that a level of cyclic acid greater than 1% in the oils would be necessary to produce toxic responses.

- b. Specific Objective: Study the effect of photosensitized oxidation on deterioration of vegetable oils.

Progress: Since normal autoxidation involving free radicals occurs concurrently with light oxidation involving singlet oxygen (an activated form of oxygen) methods were developed to distinguish between the two oxidation processes. Hydroperoxide isomers were analyzed by new methodology involving gas chromatography-mass spectrometry (GC-MS) and high-pressure liquid chromatography (HPLC). On oxidation photosensitized with methylene blue at 0 C, methyl oleate produced a 50-50% mixture of 9- and 10-hydroperoxides, linoleate a mixture of 67% conjugated (9+13) and 33% unconjugated (10+12) hydroperoxides, and linolenate a mixture of 75% conjugated (9+12+13+16) and 25% unconjugated (10+15) hydroperoxides. The unconjugated hydroperoxides of linoleate and linolenate are unique products of singlet oxygenation. The importance of photosensitized oxidation in different vegetable oil esters was demonstrated by identifying unique 10- and 12-hydroperoxides at low levels of oxidation, and by showing that singlet oxygen quenchers (carotene and tocopherol) cause a decrease of these hydroperoxides. The new GC-MS and HPLC methodology have thus provided powerful diagnostic tools to follow the extent of light oxidation occurring concurrently during free radical autoxidation of unsaturated fats.

- c. Specific Objective: Study the oxidation of isomeric dienes to determine how flavor deterioration occurs in hydrogenated soybean oil.

Progress: Two important isomeric dienes formed in partially hydrogenated soybean oil were autoxidized to elucidate the mechanism of deterioration. Oxidation at different temperatures showed that oxygen attack is favored at the terminal carbons of the 15-double bond of both the 9,15-diene and the 12,15-diene. These results may account for the "reverted" type flavor produced in hydrogenated soybean oil low in linolenic acid. The triglyceride from the 9,15-diene isomers was prepared in 100% purity for studies of the volatile compounds produced by thermal oxidation. This work is reported under item 3.

- d. Specific Objective: Study immobilized metal complex catalysts for the selective hydrogenation of soybean oil.

Progress: New polymer-bound hydrogenation catalysts were made by complexing palladium, rhodium, and nickel chlorides with anthranilic acid anchored to polystyrene, in collaboration with Dr. N. Holy, Western Kentucky University. These catalysts were active in the hydrogenation of soybean esters. The linolenate selectivity of the palladium polymer catalysts ranged from 1.6 to 3.2% and trans unsaturation from 10 to 26%. The polymeric palladium catalysts could be recycled two or three times and no palladium was detected in the hydrogenated products. This new approach may provide a way of minimizing solubilization of oxidatively detrimental and potentially toxic metals in hydrogenated oils.

Publications:

FRANKEL, E. N. Soybean Oil Flavor Stability. In "Handbook of Soybean Oil Processing Technology," edited by O. L. Brekke, T. L. Mounts and E. H. Pryde, American Soybean Association, St. Louis, Missouri, in press.

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FRANKEL, E. N. HPLC of Long Chain Hydroxy and Other Oxygenated Compounds Derived from Lipid Oxidation. Presented at the Short Course on New Aspects of Lipid Analysis, Chicago Chromatography Discussion Group and North Central Section of the American Oil Chemists' Society, Chicago, Illinois, November 19, 1979.

7. Nutritional Quality, Safety and Flavor Aspects of Soybean Protein Products (J. J. Rackis)

- a. Specific Objective: Prepare purified potato and egg white trypsin inhibitors (TI's) for longterm rat feeding studies.

Progress: Utilizing a commercial process to prepare potato starch and remove pulp, the Eastern Regional Research Center, Wyndmoor, PA, completed a large-scale preparation of potato juice concentrate, that contained 31 mg TI/g sample, compared to 36-40 mg TI/g of commercial, raw soy flour. Since 70% of the constituents in potato juice concentrate are low molecular weight substances, ultrafiltration membranes will be used to purify the TI's by BC personnel. Preparation of egg white TI is in the laboratory planning phase.

- b. Specific Objective: Identify and quantitate the amount of cyanide-containing constituents (cyanogens) in soybeans.

Progress: Under mild acid hydrolysis, approximately 0.3 μg of cyanide (CN^-)/g was found in whole soybeans, raw, defatted, and over-toasted soy flour compared to a value of 30-50 μg CN^- /g in lima beans. Our data for lima beans agrees with literature values. Values of 61 μg CN^- /g were obtained for soy flours when considerable charring occurred during strong acid hydrolysis and distillation. These results raise questions of whether CN^- is an artifact derived by the conditions of the assay method or is derived from a naturally occurring cyanogen.

- c. Specific Objective: Evaluate the role of peroxidases in the generation of objectionable flavors in soy protein products.

Progress: Soy peroxidase catalyzes oxidation of indole-3-acetic acid only in the presence of hydrogen peroxide. However, purified soy peroxidase cannot utilize linoleic acid hydroperoxide (LOOH) as a substrate for the oxidation of quaiacol or o-phenylenediamine as the hydrogen donors as evidenced by the fact that the absorption maximum at 234 nm of the LOOH did not change and that the expected reaction product, monohydroxydiene, was not present. Of considerable interest, however, was the finding that oxygen uptake occurred during incubation of purified peroxidase with LOOH. On the other hand, previous research showed that crude extracts of defatted soybean meal, containing high peroxidase and lipoxygenase activities, can utilize both linoleic and linolenic acid hydroperoxides. The significance of these divergent results with respect to enzymatic generation of flavors in soybeans needs to be evaluated.

- d. Specific Objective: Sensory evaluation of commercial soy protein products to provide a basis for improvement in the flavor of soy products.

Progress: Samples of 12 soy flours, six concentrates, nine isolates and 12 textured products have been evaluated for odor and flavor characteristics by a trained taste panel. The samples are representative of products currently manufactured by ten U.S. and two European

processors. Results show that there were few differences between the soy flours or between textured soy concentrates. Large differences in odor and flavor scores exist between concentrates, isolates, and textured soy flours. Consistently the best flavor scores were obtained with flours and particularly concentrates prepared by processes utilizing the principles of hexane:alcohol azeotrope and aqueous alcohol extraction established by NRRC research. Determination of gas chromatographic (GC) profiles of the volatile constituents of the 39 soy samples is partially completed. Multiple regression analyses will be used to correlate sensory and GC analyses.

- e. Specific Objective: Determine source of rancidity and devise methods to prevent rancidity in diets used in long-term trypsin inhibitor rat feeding studies.

Progress: Diets containing soy protein products all exhibited very good storage stability, whereas lactic casein which contains 1.4% residual fat caused diets to become rancid very quickly. In long-term feeding studies such degradative reactions could cause deleterious effects in rats. A series of 16 experimental diets with formula variables consisting of lactic casein, vitamin-free and lipid-free casein, antioxidant, added fat (corn oil and lard) and iron-type (chelated and unchelated) were prepared and stored at 24 C and 37 C. Sensory and GC analyses were made on diets removed from storage every 3 to 4 days for 1 month and then at 6 and 8 weeks. All diets containing the antioxidants, a TBHQ-BHA mixture, developed only very slight rancidity after extended storage, whereas in the absence of antioxidant, strong rancidity developed in only 4 days of storage. Since the prepared diets are placed in cold storage and periodically withdrawn for actual feeding over a 2 month period, the problem of rancidity has been successfully resolved with the addition of the antioxidant mixture.

- f. Specific Objective: Identify oxidation products of soy phosphatidylcholine molecular species and evaluate their contribution to the flavor of soy protein products.

Progress: Many oxygenated fatty acid moieties existed in phosphatidylcholine (PC) extracted from hexane-defatted soy flakes when the PC hydroperoxides were reacted with an iron-cysteine redox couple. New HPLC methods were developed to fractionate and purify the oxygenated fatty acids and to separate the (d) and (l) forms and erythro and threo isomers of epoxy-hydroxy-enes and epoxy-oxo-enes from the PC hydroperoxide molecular species.

- g. Specific Objective: Prepare low-TI soy protein isolate for longterm feeding studies.

Progress: The protocol for the evaluation of the biochemical effects of trypsin inhibitors (TI) provides for the testing of a raw, poorly digestible soy protein isolate that for practical purposes, is TI-free. Both laboratory and batch-wise pilot plant studies indicated that it was technically feasible to prepare low-TI isolates. Laboratory isolate preparations ranged from 2-8 mg TI/g compared to values of 30 and 52 mg TI/g of regular, raw soy protein isolate and raw soy flour, respectively. However, when the process was run in a commercial, continuous process pilot plant, values of 13-17 mg TI/g were obtained. The continuous on-line pH monitoring system in the pilot plant indicated that the required protein precipitation pH of 5.0-5.2 was being maintained; however, subsequent analyses at NRRC suggested improper pH control. Dispersions of the isolates had pH values of 4.70-4.85 which may account for the high TI activities found.

Publications:

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SESSA, D. J. Flavor Considerations of Soy Protein Products. *Food Prod. Dev.* 13(2) (1979):62-64.

8. Analytical and Structure Studies of Soybean Proteins (W. J. Wolf)

- a. Specific Objective: Evaluate N-terminal amino acid analysis as a possible method for quantitative determination of soybean proteins used as extenders in meat products.

Progress: Edman degradation conducted in a protein sequencer revealed that the major N-terminal amino acids of textured soy flour are glycine, leucine, isoleucine, and phenylalanine. The same amino terminal acids plus others were found in beef but in much smaller amounts. Yields of N-terminal amino acids from textured soy flour were low and variable from run to run. The carbohydrates in the sample may act as a physical barrier to diffusion of reagents and solvents during the degradation steps in the spinning cup of the sequencer.

- b. Specific Objective: Continue development of a polyacrylamide gel electrophoretic "map" consisting of a plot of electrophoretic mobilities of soybean proteins as a function of gel concentration to provide information for optimal separation of the proteins and for characterizing them.

Progress: Refinement of the gel electrophoresis method by using more porous gels has permitted analysis of soybean protein fractions which previously were found to be too large to migrate into polyacrylamide gels and therefore remained unresolved. These high molecular weight proteins, however, will migrate into agarose-polyacrylamide gels and have been separated into eight to ten bands. This improved technique now makes it possible to characterize the total soybean protein mixture in terms of their relative free mobilities and retardation coefficients which are related to net charge and molecular size, respectively.

- c. Specific Objective: Prepare lipoxygenase-1 in pure form and initiate studies on the N-terminal amino acid sequence.

Progress: Literature on lipoxygenase was surveyed and a tentative procedure has been adopted for purification of the enzyme. Preliminary gel filtration studies have been conducted with a partially purified lipoxygenase-1 obtained commercially.

Report:

WOLF, W. J. Isolation and Characterization of Neutral Lipids Associated with Soybean Protein Isolates. Presented at American Association of Cereal Chemists' Meeting, Washington, D.C., October 1979.

9. Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies (Cooperative Agreement - University of Minnesota)

- a. Specific Objective: Initiate rat feeding tests on the chronic effects of vegetable and animal trypsin inhibitors (TI's).

Progress: Experiments were begun at the University of Minnesota on September 12, 1979 with a total of 600 rats on 15 diets containing varying levels of TI activity (40-1700 mg TI/100 g diet) and dietary protein (10-30%). The animals will be sacrificed periodically and various biochemical and metabolic functions of the pancreas will be evaluated. Biopsies of the excised pancreas will be sent to Western Regional Research Center, Albany, CA, for study. In the interim, a short-term experiment designed to test the effect of dietary protein, in the absence of TI, on the pancreas was completed. Growth inhibition occurs with rats fed more than 30% casein for 4 weeks. Increasing the dietary protein level from 15 to 60% casein, accelerated secretion of pancreatic trypsin activity into

the feces threefold with a concomitant enlargement of the pancreas (hypertrophy) by the same magnitude. These results reveal that high protein diets present a significant stress on pancreatic activity in only 4 weeks of feeding.

10. Effect of Tocochromanol Dimers and Trimers on the Autoxidation of Fats
(P. L. 480 Grant - Institute of Food Science, Poznan)

Analytical and preparative methodologies involving column and thin-layer chromatography were established for the detection and separation of the various tocopherols, tocochromanols, and their dimers and trimers as mild oxidation products; a number of new dimeric and trimeric products were found and evaluated for antioxidant properties; a new natural antioxidant was isolated from cocoa shell oil; the formation of dimers and trimers was followed in several model systems; and the dimers and trimers were found to have antioxidant properties.

11. Nutritional and Physiological Studies of Soybean Hemagglutinins
(P. L. 480 Grant - M. S. University of Baroda)

Rat feeding studies are underway to determine the effect of soybean hemagglutinin on growth and on activity of intestinal enzymes. Culture filtrates of Leuconostoc mesenteroides appear to contain an inhibitor and an activator of β -N-acetylglucosaminidase and protease. The inhibitor and activator can be separated from each other by ammonium sulfate and column chromatography. When mixed together, the two fractions have no effect on the two enzymes. The inhibitor and activator appear to be glycoproteins and appear to interact with each other to abolish their individual effects.

C. TECHNOLOGIES FOR INDUSTRIAL USES - PLANT AND ANIMAL PRODUCTS

1. Chemical Modification of Soybean Oil and Its Derivatives (E. N. Frankel)

- a. Specific Objective: Prepare and characterize previously unknown hydrosilylated unsaturated fatty acid derivatives of soybean oil as part of a basic study for future technology based on renewable resources.

Progress: In order to further a fundamental understanding of the hydrosilylation of methyl oleate (an internal olefin), and of the product(s) therefrom, use of a silane other than the highly reactive chlorinated silanes is desirable. Triethoxysilane was used in catalyst studies but other commercially available silanes have been tried. These include triethyl-, trimethoxy-, diphenyl-, methyldimethoxy-, and methyl bis(trimethylsiloxy)silane. The last-named silane, with chloroplatinic acid catalyst, gave about 1% silane byproducts and over 50% silane-oleate product. Isolation

and identification of the product(s), using techniques developed for methylchlorosilane-oleate product(s) (distillation, column chromatography, crystallization) is underway. Methyl bis(trimethylsiloxy)silane will also be used in further catalyst evaluations. See also objective c below.

- b. Specific Objective: Prepare and characterize sulfur compounds of the unsaturated fatty acids of soybean oil as part of a basic study on the mechanism of sulfurization of the extreme pressure lubricant additives formerly made from sperm oil.

Progress: Characterization of crude tetrasulfides derived from soybean acids and esters is currently under investigation with the Finnegan Mass Spectrometer using chemical ionization techniques. See also objective d below.

- c. Specific Objective: Evaluate new homogeneous organometallic complex catalysts for the hydrosilylation of unsaturated fatty acids.

Progress: Seventeen homogeneous organometallic complex catalysts were evaluated for hydrosilylation of methyl oleate with triethoxysilane. The majority of these catalysts had been used successfully in hydrosilylation of terminal olefins, but there are few if any reports of their use with internal olefins such as methyl oleate. Similarly, work with internal olefins almost always uses highly reactive chlorinated silanes. Catalyst evaluation was based on gas-liquid chromatography of reaction mixtures. These analyses showed formation of low-molecular weight silane byproducts, disappearance of methyl oleate, and formation of silane-oleate products. Although none of the catalysts gave a satisfactory yield of an isolatable product, eight of them (complexes of platinum, rhodium, and iridium, most of them with triphenylphosphine ligand) showed sufficient activity to warrant further study.

- d. Specific Objective: Synthesize and characterize model organosulfur compounds to give insight into the mechanism of the sulfurization reaction and into the mode of action in the performance of extreme pressure lubricants additives.

Progress: Octadecyl tetrasulfide was synthesized and characterized by X-ray diffraction spectra in cooperation with the Naval Research Laboratory. This compound was found to crystallize in the trans,trans form, to be nonplanar and nonbranched, and to have two different kinds of sulfur-sulfur bonds. Previously only one other open-chain tetrasulfide (benzylidenimine tetrasulfide) had been reported crystallographically, and it has a cis,cis configuration. Mass, nuclear magnetic resonance, and Raman spectroscopy have been carried out on octadecyl sulfide, disulfide, and tetrasulfide.

Mass spectroscopy appears to be a good method for identification and characterization of the various sulfide species. Nuclear magnetic resonance (NMR) is a satisfactory method in characterizing sulfide species from the monosulfide to the tetrasulfide when methylene protons are adjacent to the (S)_x moiety. Raman spectroscopy works well on pure species but problems are encountered in mixtures.

- e. Specific Objective: Develop new information on microemulsions and micellar systems for hybrid fuels from both gasoline and diesel oil incorporating aqueous alcohol or other annually renewable fuels obtainable from agricultural resources as a means of conserving petroleum and energy.

Progress: Two formulations were developed for incorporating aqueous ethanol into diesel fuel and are being evaluated in diesel engine tests in cooperation with the University of Illinois. One formulation incorporates 13 volume percent of 80% ethanol into the fuel through a technique involving microemulsion formation. The second formulation incorporates 11 volume percent of 95% ethanol into the fuel through the use of a cosolvent, which may result in a detergentless microemulsion or micellar solution. Both formulations remained homogeneous to -4 F (-20 C).

- f. Specific Objective: Test the feasibility of using esters of long-chain, fatty-derived dibasic acids in solvent-free, baked-coating systems.

Progress: Dibasic acids (carboxystearic and C-21 diacid) having internal carboxyl groups with lesser reactivity (1/8 to 1/15) compared to the terminal carboxy group were partially esterified with polyols. The free carboxyl groups remaining consist mainly of the internal kind, but these have sufficient reactivity to react with film-forming resins such as hexamethylol melamine under baking conditions. Preliminary tests gave results indicating excellent flexibility and adhesion to the metal substrate for the coatings. Baked coatings from the potential new crop Vernonia pauciflora, a naturally epoxidized oil, also showed excellent adhesiveness and flexibility. Such novel coatings are conserving of petrochemical resources and also of energy, since chemical modification of the oil is not required.

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D. TECHNOLOGIES AND PRODUCTS TO INCREASE
EXPORTS OF AGRICULTURAL PRODUCTS

1. High-Temperature Soybean Cooking Oils for the Export Market (T. L. Mounts)

- a. Specific Objective: Conclude acquisition of data to characterize kinetics and mechanism of copper catalyzed hydrogenation of soybean oil in laboratory-scale pressure system for continuous hydrogenation.

Progress: Reactions were performed in the laboratory-scale pressure system for continuous hydrogenation at increasing pressures to determine the effect on copper-catalyzed continuous hydrogenation. The study included pressures up to 500 psig; the results were comparable to batch hydrogenation. Linolenate selectivity remained high at 10-12; trans formation was not affected by pressure; and conjugation was eliminated at pressures above 205 psig. Three different copper catalysts, A, a commercial catalyst; B, a commercial experimental copper; and C, an NRRC-laboratory-prepared CuSiO_2 -catalyst were studied to determine the activity of the different catalysts. B was 1.3 times more active than A and the C catalyst was 1.6 times more active than A. Data acquired in this study will provide guidance to the application of the high pressure system (5000 psig) recently acquired.

- b. Specific Objective: Study the kinetics and mechanism of continuous high pressure hydrogenation of soybean oil with copper-based and other catalysts to provide a technological basis for expanding the export market for edible soybean oil, margarine, and shortening products.

Progress: Delivery of the total high pressure continuous system was made October 1979. All electrical and steam lines were installed. Transducers for pressure, catalyst-oil slurry flow and hydrogen flow were acquired and are being tested. Plumbing of reactor flow system and sampling manifold was completed. The automatic analytical train was connected to the laboratory-scale continuous hydrogenator. The train is a Wilks Scientific infrared ATR for measuring trans, a Waters Refractometer and a Hewlett Packard GLC equipped with an automatic injector. Hydrogenations were performed using a Ni-trialkyl alumina catalyst that did not need to be filtered out of the oil before entering the chain. The system worked well except for the refractive index.

- c. Specific Objective: Develop knowledge concerning the use of Ziegler-type catalysts in batch and continuous hydrogenation of soybean oil. The goal is to improve nutritional quality of consumer products.

Progress: Reaction of copper stearate with triethyl aluminum resulted in an active soluble catalyst for selective hydrogenation of soybean oil. However the linolenate selectivity (K_{Le}/K_{Lo}) for this catalyst was much lower than that obtained with heterogeneous copper-chromite ($K_{Le}/K_{Lo} = 3$ vs 12 to 15). The activity of the catalyst was further improved when triisobutyl aluminum, trihexyl aluminum or diethyl magnesium was used instead of triethyl aluminum. Further improvement in activity resulted when silica or alumina was added to the reaction system. Also trace amounts of alcohol improved the activity while more than trace amounts destroyed the catalyst. The most active soluble copper catalyst prepared so far has an activity which is comparable to commercial nickel catalysts. Soluble nickel catalysts were prepared and successfully tested in the laboratory-scale continuous hydrogenation system.

- d. Specific Objective: Isolate and identify materials contributing to odor of soybean oil on storage and use in deep-fat cooking and salad oils.

Progress: Soybean oil was oxidized at 30 C and the volatiles collected in hexane. High-performance liquid chromatography (with hexane and micro-CN column or with aqueous ethanol and micro-C-18 column) separate the volatiles into numerous fractions. Although these fractions have distinct odors, concentrations obtained have been too low for reliable odor identification. Initial results indicate preliminary adsorption of volatiles by materials such as silica or C-18 Waters Sep-Pak may lead to higher concentrations.

- e. Specific Objective: Further clarify the understanding of which volatile compounds contribute to soybean oil room odors, to provide a basis for research to eliminate objectionable odors.

Progress: Methods were developed for the addition of volatile compounds to heated cottonseed oil. Work was initiated with the addition of acrolein to the oil. A series of saturated aldehydes, trans-2-alkenals, and trans,trans-2,4-alkadienals were obtained for addition to heated cottonseed oil.

- f. Specific Objective: Evaluate the effect of packaging on the storage stability of soybean oils for export markets.

Progress: Soybean and hydrogenated soybean oils (2 each) were packaged in two types of acrylonitrile bottles (Barex and Cycopac), clear glass and amber glass. Storage conditions included fluorescent light exposure, accelerated temperature storage and long-term ambient storage. All oils were bottled with air in the headspace. In the light exposure series, the amber glass protected the oils from deterioration, but there were few differences in quality of oils packaged in plastic and clear glass. In all tests, the oils

packaged and aged in plastic were comparable to the oils aged in clear glass bottles. The study was extended to include an evaluation of the same oils stored in PVC bottles. To complete the study, samples will be taken from storage for evaluation in February 1980.

- g. Specific Objective: Develop improved methods of cis,trans isomerization of unsaturated fatty acids to provide model compounds for research studies.

Progress: cis,trans Isomerization of unsaturated fatty acids using p-toluenesulfonic acid proved to be a convenient method. Isomerization of methyl oleate, methyl linoleate and methyl linolenate was studied. The final product from each isomer contained the equilibrium 80% total trans at each double bond.

- h. Specific Objective: Develop methods for separation and analysis of fatty acid isomers from hydrogenated soybean oil by high-performance liquid chromatography and capillary gas chromatography.

Progress: Temperature programming was applied to silver resin high performance liquid chromatography to give faster elution of methyl linoleate and to achieve elution of methyl linolenate in a practical time. This technique was applied to analysis of hydrogenated fats to separate geometrical and positional isomers and to provide fractions for further study by capillary gas chromatography. A gas chromatograph was converted from operation with stainless steel to glass capillary columns, and tested with butanediol succinate and Silar 10c glass capillaries. Factors were determined for conversion from area to mole percent by analysis of commercial standard mixtures. Equivalent chain lengths were determined for 18 carbon methyl esters to aid in identification of acids from hydrogenated fats, and the instrument was applied to analysis of such fats.

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2. Quality Soybean Oil for Export Markets (T. L. Mounts)

- a. Specific Objective: Conclude study of effects on oil quality of storing soybeans damaged by harvesting equipment.

Progress: In cooperation with AES and SEA-AR, Urbana, Illinois, soybeans were harvested at different field moisture levels and at varying speeds of the harvesting combine. The beans, with varying amounts of damage (splits), were dried to a uniform moisture (11%) and stored in on-farm silos. A total of 60 discrete samples were placed in storage and sampled at 0, 3, 7, 10 and 23 months. Soybeans were processed for crude oil recovery and critical crude oil quality analyses (iron, free fatty acid, phosphorus and peroxide value) were completed. The phosphorus content of the crude oil was significantly reduced between 10 months and 23 months storage indicating deterioration of the phospholipids had occurred. There

was no significant variation in other critical analyses. There was no correlation between the variations in harvesting conditions and variations in phosphorus analysis.

- b. Specific Objective: Develop further knowledge concerning the deterioration of oil quality during export shipment of soybeans.

Progress: Origin and destination samples from two export shipments of soybeans were received from the AES, Urbana, Illinois. Extraction and analysis of crude oil was initiated.

- c. Specific Objective: Complete design and construction of laboratory adiabatic storage silo. Study the effects of parameters of soybean storage on critical oil quality factors.

Progress: A prototype adiabatic reactor was assembled and tested for operation. Problems of insulation and external heating by fan motor were encountered; motor was remounted and insulation was improved. Tests of temperature controller showed sensitivity to line transients so electrical modifications are in progress. A testing procedure for simulating reactor temperature rise was devised and gives satisfactory performance.

- d. Specific Objective: Determine the composition and structure of the deleterious nonhydratable soybean phosphatides which are formed during soybean and oil storage and transportation.

Progress: To serve as a source of nonhydratable phosphatides, a crude soybean oil (extracted from field damaged soybeans) was degummed in the laboratory under standard conditions. Typically, a crude soybean oil will yield about 2% fluid lecithin, which is about 2/3 phosphatide and 1/3 oil. By contrast, the severely damaged crude oil yielded only 1% of a dark viscous fluid of which 34% was phospholipids. The hydratable phospholipids from the severely damaged crude oil contain the same classes of compounds as those found in commercial lecithin. Thin-layer chromatography showed the presence of 7 components including phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. Removal of the nonhydratable phosphatide from the degummed oil was attempted by solvent extraction, liquid chromatography, and acetone precipitation. All these procedures carry along considerable amounts of neutral lipids. It appears that preparative thin-layer chromatography however, can be used to isolate the nonhydratable phosphatides after preliminary separation from the degummed oil.

- e. Specific Objective: Complete study of residual metal content of commercial edible oil products.

Progress: Commercial hydrogenated vegetable oils and oil blends recovered from margarines were analyzed for chromium, copper, nickel, and iron. Samples were solubilized by warming in 50% w/v methyl isobutylketone. Apparent metal concentrations were generally at the limit of detection by graphite furnace atomic absorption analysis. Conversely, production samples taken before final refining steps contain metals; i.e., nickel, in excess of instrument range. Versatility in range of metal concentration was achieved by analysis at alternate wavelengths. Some commercial hydrogenated solid fats and blended fats for margarine formulation required a modified sample preparation procedure. Fats were mixed hot as a 20:80 blend in soybean salad oil and in turn, the blend was made 50% w/v in methyl isobutylketone. Matrix matching with the standards in tristearin complicated calculations and was judged unnecessary based upon analysis of reference lipid samples of similar hardness by direct dispersal and sampling determinations in which standards were in salad oil.

- f. Specific Objective: Study conditions that promote the formation of nonhydratable phospholipids during storage, transportation, and handling of exported soybeans.

Progress: Work was concentrated on developing suitable analytical methodology. An atomic absorption method, utilizing a graphite furnace and a electrodeless discharge lamp, was developed to give a rapid accurate method to determine elemental phosphorus. The dry, char, and atomize cycles are continuously programmed. Addition of lanthanum chloride to a solution of the oil in methyl isobutylketone was found to be necessary to enhance the sensitivity for phosphorus. The method is much faster than the AOCS standard method, i.e., analysis time was cut from a week to a matter of minutes; agreement is good. A micro method was developed to degum small samples of crude soybean oil. Oil, 30 g, along with 0.6 g (2%) water in a test tube are thoroughly mixed on a Vortex mixer. The samples are placed into oil bath maintained at 60 C. Air is used to agitate the samples. After 30 min, the foots are removed by centrifugation. The results compare well with those obtained by large-scale batch degummings.

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E. HUMAN REQUIREMENTS FOR NUTRIENTS

1. Biochemical Behavior of Isomeric Fats in Hydrogenated Soybean Oil (E. A. Emken)

- a. Specific Objective: Complete study to compare utilization in humans of cis-12- and trans-12-octadecenoic acids to cis-9-octadecenoic acid. This work will provide basic information on the nutritional impact of isomeric fats formed during hydrogenation of soybean oil.

Progress: Mixtures of triglycerides containing deuterated trans-12-, cis-12- and cis-9-octadecenoic acids were fed to two young adult male subjects and nine blood samples drawn at intervals over a 48-hr period after feeding. Each sample was separated into plasma, red cell, chylomicron and very low density, low density and high density lipoproteins. Each of these fractions was further separated into 8 to 10 individual lipid fractions, derivatized and analyzed by mass spectroscopy. In addition, platelets, triglyceride, and phospholipids from one blood sample and the fatty acids in the 1-acyl and 2-acyl position of several plasma phosphatidyl choline samples were analyzed. Selectivity values were calculated for each sample which allows the relative incorporation of the cis-12- and trans-12-octadecenoic acid isomers to cis-9-octadecenoic acid to be compared.

Results indicate the 12-octadecenoic acid isomers were nearly completely absorbed. Cholesteryl ester fractions discriminated against incorporation of the isomers and phospholipids selectively incorporated these isomers. Different selectivities for the isomers were observed for the various lipoprotein fractions. No desaturation of the 12-octadecenoic acid isomers to linoleic acid was observed. Comparison of the 12-octadecenoic acid data to trans-9-octadecenoic acid data from similar experiments indicate the 12-octadecenoic acid isomers are biologically and nutritionally more important because of their greater selectivity values and their ability to compete with linoleic acid for incorporation into phospholipids.

- b. Specific Objective: Compare the results of various synthetic schemes for the preparation of deuterium-labeled fatty acids for use in human studies.

Progress: The preparation of cis and trans-11-octadecenoic- d_6 acid isomers by synthetic schemes using the Wittig and acetylenic coupling reactions were compared. The scheme utilizing the Wittig reaction gave products with higher isotopic purity and about 30% higher overall yields. A convenient procedure was developed for the preparation of omega-acetylenic fatty acids based on the reaction of omega iodoacids with the lithium acetylene-ethylenediamine complex in hexamethylphosphoramide. The reaction was rapid and produced high yields (85%) of the omega acetylenic acid. Procedures for the synthesis of the methyl 12-oxo-dodecanoate-9,10- d_2 (12 AE- d_2) intermediate necessary for the synthesis of 12,15-octadecadienoic- d_2 acid were compared. The most facile route utilized vernolic acid (12-epoxy, cis-9-octadecenoic acid) as the starting material. This fatty acid was converted to methyl dihydroxyoleate, deuterated using Wilkinson's catalyst and then oxidatively cleaved with lead tetraacetate to yield 12 AE- d_2 .

- c. Specific Objective: Investigate novel approaches to expand the capabilities of silver-resin chromatography for purification and separation of fatty esters.

Progress: Preparative liquid chromatographic columns packed with a sulfonic acid ion exchange resin containing silver ions were prepared. These columns were used to separate a variety of polyunsaturated fatty methyl ester mixtures. Previous separations by preparative silver resin columns were limited to fatty methyl ester mixtures containing only saturated and monounsaturated esters. The new chromatographic columns contain a sulfonic acid ion-exchange resin which has only part of the available sulfonic acid protons replaced by silver ions. By controlling the amount of silver ions on the resin columns, mixtures containing dienoic, trienoic and tetraenoic fatty esters can be separated. These silver resin columns also effectively separated trans,cis- and trans,trans-, or trans,cis- and cis,cis- mixtures of octadecadienoate esters.

- d. Specific Objective: Begin synthesis of cis-9-octadecenoic-10-¹⁴C acid and complete synthesis of trans-8-octadecenoic-³H acid.

Progress: Reaction conditions were optimized for the preparation of nonanoic-1-¹⁴C acid from octylbromide and Ba¹⁴CO₃. Vacuum line procedures and reaction conditions which will be used for the preparation of cis-9-octadecenoic-10-¹⁴C acid via the Wittig coupling reaction were tested.

Methyl cis-8-octadecenoate-8(9)-³H was prepared by tritiation of the corresponding methyl octadecynoate and then isomerized to methyl trans-8-octadecenoate-³H. The resulting mixture of cis and trans isomers was purified and separated to obtain methyl trans-8-octadecenoate-³H.

- e. Specific Objective: Compare deposition of trans-12-octadecenoic acid to cis-9-octadecenoic acid in egg yolk lipids.

Progress: Analyses of yolk lipid components after feeding mixtures of radioisotope-labeled trans-12- and cis-9-octadecenoates to three laying hens was completed, including acyl positional analyses of triglyceride (TG), phosphatidyl ethanolamine (PE) and phosphatidylcholine (PC). Data show TG was the only lipid component to preferentially incorporate oleate over the trans-12-octadecenoic acid (12t-18:1) isomer. Acyl positional analysis of PE and PC showed large preferential placement of the 12t-18:1 isomer at the 1-position and discrimination against trans at the 2-position. Cholesteryl esters (CE) which usually contain cis acids preferred the 12t-18:1. Incorporation of 12t-18:1 relative to oleate indicated no preferential incorporation of 12t-18:1 at the TG(1+3) acyl position but discrimination at the TG-2 position. Selectivity

values were: PE, 0.64; PE-1, 0.81; PE-2, -0.16; PC, 0.33; PC-1, 0.84; PC-2, -0.35; TG, -0.17; TG-1+3, -0.06; TG-2, -0.21; CE, 0.21.

- f. Specific Objective: Summarize present knowledge pertaining to the formation, analysis and nutritional properties of isomeric fatty acids in hydrogenated vegetable oils.

A monograph on "Geometrical and positional fatty acids" was organized and edited for publication by the American Oil Chemist's Society. Scientific literature pertaining to the metabolism, biochemistry, nutrition, analytical techniques and formation of isomeric fatty acid isomers in hydrogenated vegetable oils was reviewed. Chapters were contributed by internationally recognized authorities in the field of lipid chemistry.

- g. Specific Objective: Compare utilization and distribution of cis-13- and trans-13-octadecenoic acids to cis-9-octadecenoic acid in young adult men. This work contributes basic information on the nutritional impact of isomeric fats formed during hydrogenation of soybean oil.

Progress: A mixture of deuterated cis-13-, trans-13-, and cis-9-octadecenoic acid was fed to a young adult male medical student and blood samples collected. Fractionation, derivatization and analysis of these samples are in progress.

- h. Specific Objective: Synthesize dideuterated geometric isomers of 12,15-octadecadienoic acid for use in human metabolism studies on the nutritional value of hydrogenated soybean oil.

Progress: A novel synthetic route was developed for the preparation of all four possible geometric isomers of 12,15-octadecadienoic-d₂ acid based on the coupling of 1-bromo-3-hexene with 12-oxododecanoic-d₂ acid. Synthesis of the cis,cis-; cis,trans-; trans,cis- and trans,trans-octadecadienoic acid isomers was possible because of techniques developed for controlling the stereochemistry of the Wittig coupling procedure and because the synthetic scheme yields only those mixtures of isomers which can be easily separated.

- i. Specific Objective: Develop improved methods for purification of reaction mixtures containing polyunsaturated fatty esters which are to be used in human metabolism studies.

Progress: The use of solvents other than methanol are being investigated as an alternative procedure for the elution and separation of polyunsaturated fatty esters by silver-resin chromatographic techniques.

- j. Specific Objective: Determine the best synthetic routes for preparation of linoleate-d₆, palmitate-d₂ and stearate-d₆ which are needed for human absorption and metabolic studies.

Progress: A versatile procedure for the preparation of deuterated palmitic acid and stearic acid was developed by extension of previous techniques used to prepare deuterated monounsaturated fatty acids. These procedures were superior to the alternate strategy of deuterating appropriate unsaturated fatty acids isolated from unusual seed oils or marine fats and oils.

- k. Specific Objective: Synthesize cis-9-octadecenoic-10-¹⁴C acid and trans-10-octadecenoic-³H acid for use in metabolism studies with the laying hen.

Progress: Sufficient cis-9-octadecenoic-10-¹⁴C acid (5.7 mCi) was synthesized for use in several laying hen studies. The Wittig coupling reaction was employed to prepare the above product.

- l. Specific Objective: Identify volatiles formed during heating trilinolenin and tri(cis-9, cis-15-octadecadienoyl) glycerol.

Progress: The mass spectra of volatiles produced during heating of linolenic acid and cis-9, cis-15-octadecadienoic acid triglycerides have been obtained and are being interpreted.

- m. Specific Objective: Determine accuracy and sensitivity of chemical ionization and electron impact ionization of the Finnigan mass spectrometer for analysis of mixtures of deuterated fatty esters and deuterated fats incorporated into human blood lipids.

Progress: Preliminary investigation of a mixed ionization gas (methane and isobutane) for chemical ionization selected ion monitoring mass spectrometry showed a ten-fold improvement in sensitivity over electron impact ionization techniques.

- n. Specific Objective: Initiate cooperative research with St. Francis Medical Center, Peoria, IL on determination of positional fatty acid isomers in human tissue lipids.

Progress: Protocols for obtaining medical and dietary histories of subjects and procedures for selection of subjects and tissues have been prepared. Techniques for homogenizing various tissue types have been tested. Validation of methods for extraction, fractionation and quantitation of isomeric octadecenoic acids in tissue lipids has been made.

- o. Specific Objective: Identify molecular structures using gas chromatography-mass spectrometric analysis in collaboration with both scientists from within and outside NRRC.

Progress: Interpretation of mass spectra obtained for several unknown compounds is in progress.

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